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Neuropsychological Deficits and Cerebral Lesions in Mild Traumatic Brain Injury

Irina Andreea Radoi

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Irina Andreea Radoi

Joan Sahuquillo Barris
M. Antonia Poca i Pastor

Directors

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Foreword

The present doctoral thesis has been conducted in the Neurotraumatology and Neurosurgery Research Unit (UNINN: ***Un**idad de **I**nvestigación de **N**eurotraumatología y **N**eurocirugía*). UNINN is a consolidated research group acknowledged by *Agència de Gestió d'Ajuts Universitaris i de Recerca de la Generalitat de Calunya* (2017 SGR 975) and part of the Vall d´Hebron Research Institute (VHIR). This doctoral thesis has been funded by *Fondo de Investigación Sanitaria* (*Instituto de Salud Carlos III*) with grant FIS PI13/02397, which was co-financed by the European Regional Development Fund (ERDF) and awarded to Dr. M. A. Poca, and by the VHIR with a personal pre-doctoral grant (PREDVHIR-2012-26). Financial support has also been received from *Fundació Mapfre* through grant 2012-04, awarded to Dr. M. A. Poca. The funders had no role in study design, data collection, data analysis, data interpretation, or the writing of this work.

This thesis, presented for the degree of Doctor by *Universitat Autònoma de Barcelona*, follows the published papers format and includes two published peer-reviewed papers. The studies are the following:

1) Rădoi A, Poca MA, Cañas V, Cevallos JM, Membrado L, Saavedra MC, Vidal M, Martínez-Ricarte F y Sahuquillo J. *Alteraciones neuropsicológicas y hallazgos neurorradiológicos en pacientes con conmoción cerebral postraumática. Resultados de un estudio piloto.* Neurología. Sep 2018; 33(7): 427—437. (IF 2,038).

This study was originally published in Spanish. By editorial decision, it was later published in the English version of the journal. The English version is included in the Supplementary Material.

2) Rădoi A, Poca MA, Gándara D, Castro L, Cevallos M, Pacios ME y Sahuquillo J. *The Sport Concussion Assessment Tool (SCAT2) for evaluating civilian mild traumatic brain injury. A pilot normative study.* PLoS ONE. Feb 2019; 14(2): e0212541. (IF 2,776)

Relevant data produced by this thesis and that have not been published are presented in Supplementary Material, together with a third paper, currently in preparation:

Rădoi A, Poca MA, and Sahuquillo J. *Is it possible to screen for patients at high risk of developing postconcussive syndrome? Results of a pilot study using serum biomarkers and clinical variables* (in preparation)

The results of this project have also been publicly presented at international events, through oral and poster communications, as follows:

Oral communications:

"Endorsement of Cognitive Postconcussional Symptoms and Neuropsychological Functioning in Mild TBI. A Pilot Study" at the American Congress Rehabilitation Medicine 93rd Annual Conference. Progress in Rehabilitation Research (Chicago, USA, 2016)

"*El paciente olvidado: el TCE leve*" [The forgotten: patients with mild traumatic brain injury] at the *XVII Simposium Internacional de Neuromonitorización y Tratamiento del Paciente Neurocrítico* (Barcelona, Spain, 2016)

"Marcadores biológicos y neuropsicológicos tempranos del traumatismo craneoencefálico leve y su pronóstico" [Early biological and neuropsychological markers of mild traumatic brain injury and its prognosis] VI Congreso Nacional de Neuropsicología (Málaga, Spain, 2013)

Poster presentation

"Subjective endorsement of cognitive postconcussional symptoms in a cohort of mild TBI patients. A pilot study" at the International Neuropsychological Society 2016 Mid-Year Meeting. From Neurons to Neurorehabilitation (London, UK, 2016)

Glossary of Abbreviations and Acronyms

ACRM	American Congress of Rehabilitation Medicine
ANKK1	ankyrin repeat and kinase domain containing 1
APOE	apolipoprotein E
ATP	adenosine triphosphate
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BM	biomarker
BMI	body mass index
BVMT-R	Brief Visual Memory Test – Revised
Casp-1	caspase 1
CDE	Common Data Elements
COMT	catechol-o-methyltransferase
CPT	Conners' Continuous Performance Test
CSF	cerebrospinal fluid
CT	computed tomography
CTE	chronic traumatic encephalopathy
DAI	diffuse axonal injury
DDT	Delay Discounting Test
DNA	deoxyribonucleic acid
DRD2	dopamine receptor D2
DTI	diffusion tensor imaging
ED	emergency department
EI	Evans' index
ELISA	enzyme-linked immunosorbent assay
FLAIR	fluid-attenuation inversion recovery
FU	follow-up
GCS	Glasgow Coma Scale
GFAP	glial fibrillary acidic protein
GRE	gradient recall echo
HADS	Hospital Anxiety and Depression Scale
ICoMP	International Collaboration on mTBI Prognosis
IL-1	interleukin 1
Lep	leptin
LOC	loss of consciousness
mBESS	modified Balance Error Scoring System
MP-RAGE	magnetization-prepared rapid gradient echo
MRI	magnetic resonance imaging
mTBI	mild traumatic brain injury
NINDS	National Institute of Neurological Disorders and Stroke
NSE	gamma-specific enolase
NVU	neurovascular unit
OI	orthopedic injury
PAR	poly-ADP-ribosylation
PARP	poly(ADP-ribose) polymerase
PCE	potential concussive event
PCS	post-concussion syndrome
PPCS	persistent post-concussion symptoms
PTA	post-traumatic amnesia

PTSD	post-traumatic stress disorder
RMiET	Reading the Mind in the Eyes Test
RPQ	Rivermead Post-concussion Questionnaire
SAC	Standardized Assessment of Concussion
SCAT2	Sport Concussion Assessment Tool 2nd Edition
SD	standard deviation
SNTF	calpain-derived αII-spectrin N-terminal fragment
SRC	sports-related concussion
SWI	susceptibility-weighted imaging
TAP	*Test de acentuación de palabras*
TBI	traumatic brain injury
TCVI	traumatic cerebral vascular injury
TMT	Trail Making Test
ToL	Tower of London
TOMM	Test of memory malingering
UCH-L1	ubiquitin C-terminal hydrolase-L1
VEGF-A	vascular endothelial growth factor A
vWF	von Willebrand factor
WAIS-III	Weschler Adult Intelligence Scale 3rd Edition
WCST	Wisconsin Card Sorting Test
WHO	World Health Organization
WM	working memory
WMHI	white matter hyperintensities

Synopsis

Mild traumatic brain injury (mTBI) accounts for around 80% of all traumatic brain injuries. Concussion is commonly regarded as a synonym for mTBI. In the last two decades, concussion has been a topic of public interest, especially in the United States, that has triggered a reassessment of the healthcare community towards the mTBI-concussion spectrum. There is currently no gold standard for concussion diagnosis.

Concussion has been traditionally considered a fundamentally reversible syndrome, understood as a physiological alteration lacking any structural brain injury. This view has been challenged by evidence exposing structural damage, in particular axonopathy, and in vivo metabolic dysfunction that does not recover as quickly as the symptoms. Furthermore, multiple reports on persistent and debilitating cognitive deficits and psychiatric symptoms manifesting even years later also questioned the reversibility of the post-concussion symptoms. Nonetheless, the lack of biomarkers sensitive and specific enough to brain injury has not only contributed to the ongoing confusion on diagnosis but made tracking recovery difficult. The identification of reliable diagnostic and prognostic biomarkers for mTBI has remained a major unmet clinical need.

The aim of this thesis was to characterize, from various viewpoints, the early state of patients who sustained an mTBI and its delayed repercussions and ultimately to provide evidence useful for the early screening of patients at risk of an unfavorable outcome following concussion. In addition to clinical data, the protocol comprised blood biomarkers (proteins and genetic variants), objective cognitive descriptors and patient-based outcome measures; in a subgroup, the information available on routine computerized tomography [CT] scan was contrasted with magnetic resonance imaging.

Patients were recruited prospectively in the emergency department (ED) of Vall d'Hebron University Hospital, a tertiary trauma center in Barcelona. Consistent with the traditional concussion criteria, normal neurological status upon examination in the ED and no abnormalities visible on the CT-scan were required for recruitment. Blood sampling was performed as soon as possible during their ED stay. They were evaluated three times in terms of symptomatology: as

soon as possible during their ED stay and always in the first 24 hours post-injury, and 1-2 weeks and 3 months later. The last two follow-up visits included a broad neuropsychological assessment.

Eighty-nine patients selected between 18 and 65 years old, without neurological or psychiatric history, and who presented symptoms of a concussion in the first 24 hours after mTBI, were recruited between April 2013 and April 2017.

Data on the subsample recruited in the first year of the study was published in a pilot study. During that year, we registered all consecutive cases that attended our centre with mTBI. The first remarkable finding of that stage of the project was that after screening more than a thousand patients, we were able to recruit less than 5%, due to the strict exclusion criteria imposed with the purpose of limiting confounding effects (mostly on the cognitive scores or protein values). The interpretation should be put into a broader context of mTBI population.

Results of the pilot study included some cases with lesions visible by MRI (despite normal admission CT scan), particularly microbleeds. Moreover, the neuropsychological data showed that, at 1-2 weeks after mTBI, generalized cognitive deficits were noticeable, with memory levels showing the biggest difference to the control group.

Fundamentally our project was aimed at identifying biomarkers of brain injury following concussion. But, at the same time, the study of biomarkers should be accompanied by considerable effort put into improving the evaluation of outcome. Self-reported symptoms have become the most used outcome measure following mTBI. In addition, the early higher symptom burden has been associated with delayed recovery and to a more intense symptomatology on the longer term. Currently, symptom assessment is an essential part of the concussion examination. Nevertheless, the criteria regarding PCS, the definition of clinically relevant PPCS are highly inconsistent. Therefore, we decided to apply a data-driven approach to the identification of "abnormal" values in concussion symptom presentation. This strategy could set aside any arbitrary threshold.

In a second study, we presented that a cohort of 60 healthy adults with no history of TBI or any other relevant known health issue frequently endorsed concussion-like symptoms, up to a point where 58.3% would be classified as presenting PPCS based on reporting 3 symptoms or more. Participants underwent brain MRI, which enabled us to confirm that the elevated concussion-like symptoms were not explained by any brain pathology.

Further examination, that is presented in the Supplementary Material, established that the expression of S100b, VEGF-A and C-reactive protein was increased in the first 24h after mTBI in comparison with reference levels. These results form part of a third article, which is currently in preparation, that looks at possible predictive value of the protein biomarker panel for experiencing persistent post-concussional symptoms.

Global analysis on the neuropsychological data showed that, in this cohort, the cognitive alterations did not persist at 3 months after mTBI. However, 22 of 46 cases (47.9%) still referred at least 3 post-concussion symptoms. It is worth adding that attrition is one of the most important limitations on the results of this study, including the evaluation of neuropsychological data.

Furthermore, the results point to a modulation of the post-concussion memory performance by the polymorphism of gene ANKK1.The functional variants of the gene PARP-1 could also play a part, as suggested by results observed on memory status at 3-months following mTBI (but not at 1-week). However, the PARP-1 polymorphism was not a significant predictor in models where it was accounted for ANKK1.

Considering these results, several directions of improvement in the mTBI clinical management and the utility of including symptomatology-based tools in the long-term outcome assessment become apparent. Currently, examination relies on a neurologic exploration and, in most cases, on performing a CT scan. In view of multiple markers of brain injury (protein and MRI-based), the conclusion on the basis of normal CT findings that a particular mTBI is a banal incident is unsupported. In addition, early routine examination could benefit from the inclusion of tools as SCAT2, that achieve a systematic assessment of early symptoms and their severity together with other signs and indicators of concussion.

I. Introduction

> *"Given the imperfect accuracy of state-of-the-art assessment in mTBI, there is significant risk for both overdiagnosis and underdiagnosis of mild traumatic brain injury and postconcussive symptoms. Both types of errors may result in human suffering."*
>
> (James F. Malec,
> in a Letter to the Editors of ACRM, March 1997)

1. Defining traumatic brain injury

Traumatic Brain Injury (TBI) is an insult to the brain induced by a mechanical external force applied to the head and can lead to long-lasting physical, cognitive, emotional and behavioral impairment. TBI maintains its global position as the first cause of mortality among young adults and is a major cause of morbidity across all ages (Maas et al., 2017). In the last 50 years, diagnostic and treatment guidelines have broadly distinguished between three heterogenous categories of clinical severity: mild, moderate and severe TBI. By all criteria in use, more than 80% of cases are mild TBI (mTBI). In the European Union, it is estimated that 57,000 TBI-related deaths and 1.5 million TBI-related hospital admissions occur every year (Majdan et al., 2016). Hospitalization, treatment and rehabilitation costs represent only a fraction of the societal burden of TBI. Permanent sequelae and devastating loss of quality of life affect a frequently young group of patients. Labor and productivity losses are aggravated by the complex impact of this condition on family members. TBI triggers an increased risk of long-term complications such as epilepsy, dementia or stroke and can exacerbate pre-existing comorbidities. Even the most recent estimates of years of life lived with disability after TBI do not fully take into account long-term neurological and psychological sequelae of TBI (James et al., 2019).

In order to improve epidemiological data and case ascertainment, various organizations have posited definitions for TBI, including the American Congress of Rehabilitation Medicine (ACRM) (Kay et al., 1993), the Centers for Disease Control (Faul, Xu, Wald, & Coronado, 2010) and the World Health Organization (WHO) (Holm, Cassidy, Carroll, & Borg, 2005). One of the consensus definitions comes from the National Institute of Neurological Disorders and Stroke and states that TBI is "an alteration in brain function, or other evidence of brain pathology, caused by an external force"(Menon, Schwab, Wright, & Maas, 2010). Therefore, TBI embodies the type of acquired brain injury in which mechanical forces induce damage to the brain in an "evident" manner (i.e. visible, either macroscopically or with neuroimaging techniques) or that manifests itself through "an alteration in brain function". Evidence of brain pathology can include visual, neuroradiologic or laboratory confirmation on damage to the brain. The alteration of brain function can be defined by any of the following: loss of consciousness (LOC), post-traumatic amnesia (PTA), neurological deficits or any alteration in mental state at the time of the injury (confusion, disorientation, slowed thinking, etc.). In all definitions, the presentation associated with TBI excludes displays related to drugs or other psychoactive substances, caused by other injuries or their treatment (e.g., systemic injuries, facial injuries or intubation) and caused by other problems (e.g., psychological trauma, coexisting medical conditions).

1.1. Causes, mechanisms and types of injury

TBI has been deemed as the most complex injury in the most complex organ of the body, considering the tremendous heterogeneity in causal forces, the pattern and extent of damage they induce and the intricate unfolding of the brain's response to insult. Various criteria are used to classify TBI into categories that share certain etiological and pathophysiological characteristics and, in theory, benefit from similar therapeutic approaches.

To begin with, the head injury is described as closed or open, on condition of the integrity or, respectively, penetration of the *dura mater*, the outermost meninx surrounding the parenchymal tissue. Secondly, the cause of injury is informative about the type, intensity and duration of the external forces involved and therefore it is advantageous to separate between ground-level falls, motor vehicle accidents and blasts among other etiological categories. In the last decade, as Europe population has been ageing, ground-level falls, that occur more frequently among the elderly, have become the most prevalent injury scenario. Meanwhile, in developing countries, the increase of motorized vehicles has been associated with a rise of traffic-related TBI cases. In high-speed vehicle accidents, the deceleration forces induce immediate neural shearing and trigger progressive axonal injury (Gennarelli & Graham, 2005). By contrast, in fall-related accidents or blows to the head, injuries involve the bruising of the brain against the skull and the development of contusions, especially in the orbitofrontal cortex and the inferolateral and polar temporal areas.

TBI is not an isolated event, it behaves as a chronic disease process that can evolve over months, years or even the course of a lifetime (Forslund et al., 2019; Masel & DeWitt, 2010). The damage inflicted at the time of the injury represents the primary damage and it is essentially unpreventable. It includes fractures, contusions, bruises, shearing of white-matter tracts and vascular tearing. The host's response to these early neurotrauma events triggers a cascade of neurochemical changes that can progress over days and leads to secondary injuries such as hypoxia-ischemia, swelling, raised intracranial pressure and infection. The secondary insults are shaped by neurotransmitter release, free-radical generation, calcium-mediated damage, gene activation, mitochondrial dysfunction, and inflammatory responses, among others (Maas, Stocchetti, & Bullock, 2008).

Further into the pathophysiological distinctions, the pattern of damage to the brain parenchyma visible on neuroimaging can be focal (hematoma, contusion, laceration), or multifocal; the latter is also called diffuse brain injury, despite its use being misleading in cases where there are multiple lesions, but not necessarily widely distributed. Under the diffuse brain injury category fall diffuse axonal injury (DAI), diffuse vascular injury (multiple microhemorrhages) and hypoxic-ischemic changes. However, it is worth noticing that, although in some cases the lesion identified is focal, the extent of damage to the brain always goes beyond its visually identified discrete boundaries and, up to some extent, focal and diffuse lesions always coexist (Bigler, 2001).

The most frequently used classification system for neuroimaging findings in acute TBI remains the Marshall classification scale, although it has been proposed nearly three decades ago and it has some limitations (Marshall et al., 1992; Pargaonkar, Kumar, Menon, & Hegde, 2019). It describes six degrees of severity based mostly on diffuse swelling and the volume of focal lesions. The higher categories are associated with increased risk of poor global outcome, neuropsychological impairment and complications such as intracranial hypertension (Mataró et al., 2001; Poca et al., 1998). Specifically, it distinguishes between four types of diffuse injuries and it is worth noticing that the type I diffuse injury is not visible on CT scan (**Fig 1**). Furthermore, the lack of evidence of brain injury indicates an apparently normal brain structure but keeps no connection with the clinical severity of the case. As a matter of fact, DAI has been established as the pivotal lesion in comatose TBI patients with poor evolution and normal early CT findings (Sahuquillo & Poca, 2002).

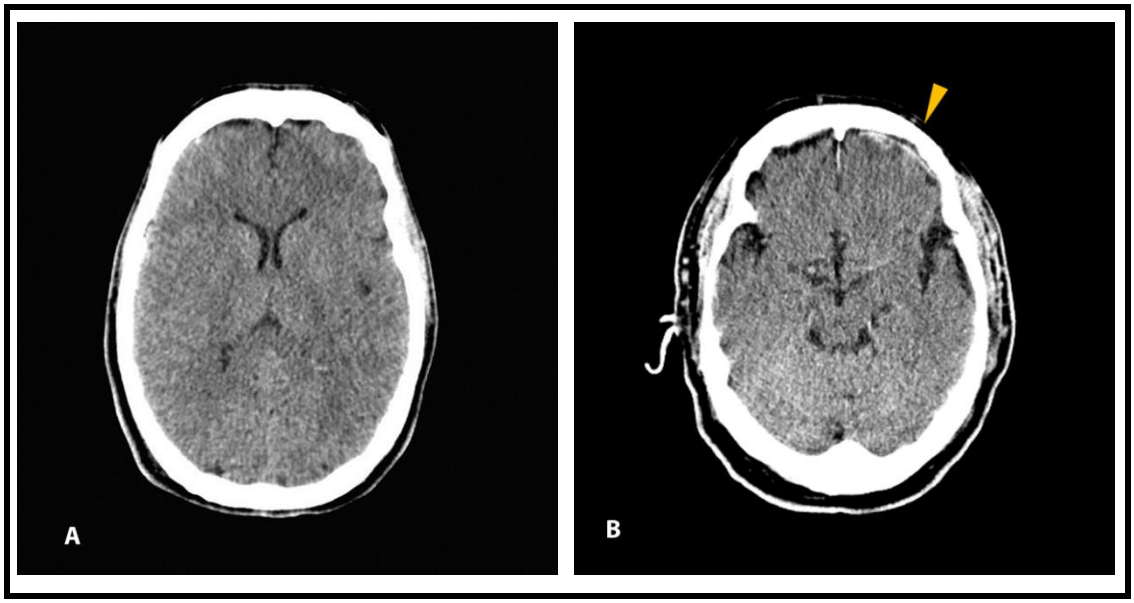

Fig. 1. Examples of CT scan results in TBI and their corresponding severity score on the Marshall classification scale. In **image A,** normal CT scan findings (Diffuse injury, Grade I) observed in a 19-year old patient at 3 hours after a moderate TBI (initial GCS score of 10), after being involved in a road traffic accident and ejected 4m away from the passenger seat through the windshield. In **image B**, a small contusion is visible in the right frontal lobe (arrowhead, Diffuse injury, Grade II) of a 69-year old man that had a GCS score of 15 at all time after hitting his head occipitally in a casual ground-level fall. GCS: Glasgow Coma Scale; TBI: traumatic brain injury.

2. Mild traumatic brain injury and concussion

2.1. The mild end of the TBI severity spectrum

Despite its wide severity spectrum, TBI is clinically classified into one of only three categories–mild, moderate and severe–by a gross evaluation of the patient's early neurological status following the insult. This assessment is most commonly performed with the Glasgow Coma Scale, as it has shown better inter-rater agreement tan other similar tools (Teasdale & Jennett, 1974; Teasdale et al., 2014). Each of the three components of the scale is assessed by a standardized approach (**Table 1**) and summed up in a score that varies between 3, when the person lacks any sort of response to stimuli and is completely unconscious, and 15, in fully conscious states. Traditionally, according to the GCS score, the TBI is considered mild between 13 and 15, moderate between 12 and 9, and severe between 8 and 3.

Table 1. The components of the Glasgow Coma Scale (GCS) and their scoring

Best motor response	Verbal response	Eye opening
1 None	1 None	1 None
2 Extension	2 Sounds	2 To pressure
3 Abnormal flexion	3 Words	3 To speech
4 Normal flexion (withdrawal)	4 Confused	4 Spontaneous
5 Localizing	5 Orientated	
6 Obeying commands		

In clinical practice, GCS scoring is frequently an irreplaceable procedure performed in TBI assessment and it is considered sufficient before classifying a case as mild, moderate or severe TBI. However, despite being the most frequently used criterion, the literature consensus has been to use the CGS together with two clinical indicators: LOC and the duration of PTA (**Table 2**). PTA is a temporary stage of confusion that occurs immediately following TBI, in which the person cannot recall events happening before or, more often, after the injury. PTA can last for minutes, hours or days and is resumed when the person can continuously form memories and retain them adequately (Roberts, Spitz, & Ponsford, 2016). The TBI severity is given by the poorest result in any of the three criteria assessed. Accordingly, a patient with a PTA that exceeds 24 hours should be considered a moderate TBI even if the GCS score remains between 13 and 15. By all criteria in use, the vast majority of the cases, between 70 and 90%, are classified as mild TBI (mTBI) (Maas et al., 2017).

This classification is strongly related to post-traumatic mortality. Severe TBI is associated with a mortality estimated around 30–40% and moderate TBI, around 15–20%. Some studies report figures as high as 8% of mortality associated with mTBI, but they systematically excluded the milder cases from their TBI series and included, for example, only TBI patients with GCS 14 or less, with abnormal CT findings or that required hospital admission (Teasdale et al., 2014). More recent evidence is also inaccurate, because the reports do not distinguish between death due to mTBI, to extracranial concurrent injuries or unrelated to the injury (Carroll et al., 2014). All in all, mTBI is estimated to be directly associated with a mortality of less than 1%.

Table 2. Clinical criteria of TBI severity, according to the American Congress of Rehabilitation Medicine (Kay et al., 1993)

	Severe	Moderate	Mild
GCS score	3–8	9–12	13–15
Duration of LOC	> 60 min	30–60 min	< 30 min
Duration of PTA	> 7 days	1–7 days	< 24 h

GCS: Glasgow Coma Scale; LOC: Loss of Consciousness; PTA: post-traumatic amnesia.

Furthermore, the early severity of TBI is also a moderate predictor for global outcome, in terms of disability vs good recovery. There was a widely held belief that the mortality gradient was broadly reflective of the risk for relevant post-traumatic sequelae associated with each TBI severity. After a severe TBI, up to 60% of the survivors have a poor outcome, a category that includes patients in a minimally consciousness state or with severe disability (Rosenfeld et al., 2012). An estimated 35% of patients with moderate TBI achieve the same unfavorable results (Watanitanon et al., 2018). In the mild category, soon after the GCS classification was put in use, reports on the unsatisfactory recovery of a considerable percentage of individuals with mTBI were published (Rimel, Giordani, Barth, Boll, & Jane, 1981). However, because good outcome was rapidly achieved by 85% of the "more severe" mTBI cases–with aggravating complications-, the recovery of cases without risk factors was never a problem of clinical interest.

The assumption that mTBI is not a benign event and can have lasting and perhaps irreversible consequences, has been replicated many times, albeit with limited echo in the clinical community. For decades, the clinical and research neurotrauma community has focused on severe TBI, inevitably at the expense of other patient groups. Given the worldwide continued high incidence of all-severity TBI (in the U.S., between 180–250 cases per 100,000 habitants per year, and in Europe 248 cases on average (Brazinova et al., 2015)), the undebatable high percentage of cases ascribed to the mTBI category make this condition a societal and public health concern. Despite a diminished individual impact, the mTBI category has the highest contribution to the global burden of disability following TBI (Te Ao et al., 2015). Even more so, there are reasons to believe that mTBI is underreported and underdiagnosed, in particular because of individuals not presenting to the hospital after injury and other cases with more severe systemic insults failing to receive proper head injury assessment. These issues were addressed by a population-based study on TBI conducted in New Zealand, referred as the BIONIC study, that actively recruited cases using both prospective and retrospective surveillance systems for 1 year (Feigin et al., 2013). The BIONIC study group concluded by estimating an annual incidence rate of 790 per 100,000 habitants, out of which 749 were mTBI (94.8%), both much higher estimates than generally seen in epidemiological studies. If these numbers are replicated, the need to produce robust data on this particular entity, including its epidemiology, pathophysiology, diagnostics peculiarities, disease course and best available treatment is even more stringent that thought.

2.2. Boxers, veterans and athletes hit their head

Since the 1920s, it became popular culture that after taking considerable blows to the head boxers were affected by a type of dementia, called punch-drunk syndrome or *dementia pugilistica* (Martland, 1928). This was considered an isolated condition among boxers, that did not concern other athletes. They encountered subtle cognitive, psychological and behavioral changes that progress over time and became severely debilitating. For a long time, the clinical decline was not associated with the history of mTBI because it was deemed too remote. However, mTBI has been

acknowledged as a topic of intense public concern in the last 15 years (Maas et al., 2017; Spira, Lathan, Bleiberg, & Tsao, 2014). The public interest in the long-term repercussions following mTBI raised exponentially, after extensive media coverage of high-profile sportsmen who suffered from severe mood and behavioral problems and committed suicide, raising doubts about the similarities with the boxer's dementia. At the same time, there were alarming military reports on mental health associated with mTBI following deployment in Afghanistan and Iraq. On the one hand, in many team and combat sports, blows to the head accompanied by transitory alterations of consciousness, and even LOC, are frequent events. On the other hand, 20% of the veterans of Operation Enduring Freedom and Operation Iraqi Freedom have a clinical diagnostic of mTBI (Cook & Hawley, 2014). The substantial volume of data that presented sportsmen and veterans suffering cognitive and psychiatric symptoms, even years following their traumatic incidents, has boosted the interest from the neurotrauma community in the mTBI pathology. However, this approach led to having most literature published in the last ten years in the field of mTBI rooted in sports and military medical communities. These studies introduce important hypotheses concerning any mTBI occurrence, but their straightforward applicability to the general population is not warranted due to considerable distinctions among them. Firstly, sportsmen are adolescents or young adults, in good general health, whose impact to the head can be accurately recorded and whose immediate post-traumatic status can be assessed by a trained observer but who are frequently subjected to repetitive mTBI. Also, athletes may try to tamper with assessments in an attempt to return to play sooner. Secondly, in military operations, the conditions of war lead to a distinct mTBI pathophysiology when explosive forces are involved in the injury mechanism (referred to as blast mTBI). Retrospective case identification, high numbers of polytrauma and high incidence of post-traumatic stress disorder (PTSD) also shape the results of mTBI studies with veterans. Noteworthy, the members of both these communities undergo thorough assessments and their general health, as well as psychiatric and neurologic pre-injury state can be objectively taken into consideration when assessing mTBI presentation and outcome. The third group of studies focus on patient groups, which are typically comprised of attendees of emergency departments (EDs). The clinical groups are the most heterogenous in terms of preinjury health status, socioeconomic and education levels, etc.

It is worth mentioning that, despite the traditional particularities detailed above, future studies could focus on the commonalities of mTBI between samples, as a recent report showed that between 2001 and 2017, 86% of mTBI in the military occurred in day-to-day activities, unrelated to deployment (NCAA-DOD Grand Alliance, 2019). Studies selecting impact mTBI (not blast-induced) and unrelated to deployment could be very valuable for civilian care.

Another consequence of having reports surging from distinct settings is a lack of consistency in the terminology regarding the insult, as the sports-related publications and much of the public conversation generated were using the term concussion. The medical literature treated the mTBI in line with the belief that it embodies the traumatically-induced brain dysfunctions at the mild end of the severity spectrum. Although concussion existed in the medical lexicon since

Hippocrates, it referred to a distinct clinical not neuropathological entity (McCrory & Berkovic, 2001). Meanwhile, the military reports used mTBI and concussion interchangeably (Management of Concussion/mTBI Working Group, 2009). Up to this date, many experts consider the two equivalent (Levin & Diaz-Arrastia, 2015; Maas et al., 2017), but others warn about various sources of confusion that arise from this (Mayer, Quinn, & Master, 2017; Sharp & Jenkins, 2015). Historically, a concussion was defined as an essentially reversible syndrome, explained by a physiological brain alteration that took place in the absence of brain structural disruption. Because of this, it was assumed that any acute symptoms would resolve spontaneously within days. In 2009, the Veteran Affairs and Department of Defense Working Group, despite considering the two terms equivalent, made the recommendation of using concussion when treating with patients, hoping that would reduce concern and stigma associated with the label "brain injury"(Management of Concussion/mTBI Working Group, 2009). More recently, many clinicians recounted (albeit anecdotally) that, in response to the public conversation that has been taking place, the stigma and the diagnostic threat had passed on to the more informal term; at the moment, their patients fear more the diagnosis of concussion than that of mTBI.

2.3. Is concussion the mildest form of mTBI?

Distinct emotional connotations and preference between medical specialties are not the most worrying issues regarding the terminology (Sussman, Pendharkar, Ho, & Ghajar, 2018). One topic is that some authors consider concussion a less severe form of mTBI, excluding the cases that show abnormalities on standard structural neuroimaging studies but without settling any clear delineator between the two. Moreover, the same criterion is used to separate between complicated (with visible abnormalities on computer tomography [CT] scanning, i.e. CT+) and uncomplicated mTBI (without neuroradiological findings, i.e. CT-), leaving aside the association with concussion (Hasan et al., 2014; Lange et al., 2012; Ponsford, Cameron, Fitzgerald, Grant, & Mikocka-Walus, 2011).

A second debated view postulates that concussion is a form of mTBI, while the converse is not true and therefore the terms should not be used interchangeably (McCrory, 2001). This argument puts together historical considerations and appears to stand to recent scrutiny, as there is no data to date that directly contradicts it. Concussion is a clinical syndrome whose essential element is the transient alteration of brain function (with various ways of identification). At the same time, the development of the mTBI entity that followed the introduction of the GCS has also been intrinsically based on clinical presentation, but it has come to incorporate a lot of data that could be not applicable to concussion, when lacking any connection with the clinical profile. Under this conceptualization, concussion is defined as the manifestation of a type of mTBI and not automatically reflective of its severity (Tagge et al., 2018). There is no denying in the neurobiological basis of concussion, but the comprehension of its underpinning neurotraumatic

mechanisms should not be separated from the profile of clinical reversible symptoms (McCrory & Berkovic, 2001).

Clearly, there is no consensus on the demarcation between concussion and mTBI. This makes impossible to distinctly position these entities in relationship with chronic traumatic encephalopathy (CTE), repetitive injury, potential concussive event (PCE) and post-concussion syndrome (PCS). CTE is the nosological entity that has replaced *dementia pugilistica*; it is a histopathologically-defined neurodegenerative disease that lacks clinical definition criteria (McKee et al., 2016), but is associated with repetitive mTBI and putatively even PCEs, on a dose-dependent response curve. PCEs or subconcussions are impacts to the head that do not produce a concussion, although it remains debated whether these incidents should be labeled as mTBI cases or are too mild to enter the "diagnostics-worthy" brain injury spectrum. It is also not clear if a single mTBI or an isolated concussion can lead to CTE (Goldstein et al., 2012; Iverson, Luoto, Karhunen, & Castellani, 2019). While the label repetitive injury is self-explanatory, the implications of suffering a secondary concussion/mTBI are complex and pathological changes induced by an insult in an unrecovered brain are not merely additive (Dashnaw, Petraglia, & Bailes, 2012). PCS is an umbrella term with inconsistent diagnostic boundaries describing a complex constellation of symptoms that are displayed after a concussion and do not recover as soon as expected (Prigatano & Gale, 2011; Reuben, Sampson, Harris, Williams, & Yates, 2014). Although by definition it is linked to a concussive event, PCS has been intensely studied in relation to mTBI too (Hou et al., 2012).

3. mTBI presentation and routine evaluations

In the clinical setting, there is significant controversy on how to diagnose mTBI and concussion. The criteria exposed above, in **Table 2**, fall short of a genuine operative definition for mTBI. In a study that applied 17 different mTBI definitions for the classification of a cohort of 11,907 children aged 3 to 16 years, the percentage diagnosed with mTBI varied from 7.1% to 98.7% (Crowe et al., 2018). The contemporary lack of agreement between diagnostic criteria can only be overcome by using reliable indicators of clinical display and subjacent brain injury.

3.1. The TBI approach: focus on risk factors for intracranial injuries

Currently, the overwhelming majority of concussion patients do not present with evidence of brain injury at routine examinations. In mTBI, CT scanning, the neuroimaging technique of choice for the study of neurotrauma lesions, generally yields normal results. The minority of mTBI cases that display post-traumatic changes on CT imaging is estimated at less than 9% (Haydel, 2015) and can even be as low as 1–3% (Stiell et al., 2005). The use of this technique is disputed in

routine evaluations of mTBI due to questionable cost effectiveness and safety considerations, raised especially for children. Moreover, in one series of 321 patients presenting with CT-identified intracranial hemorrhage after mTBI, only 4 (1%) experienced neurological decline and another 4 patients required neurosurgical intervention (Washington & Grubb, 2011). These results support that the added value of CT study in mTBI management is minor, despite being indispensable in moderate and severe cases.

There is increasing evidence that up to 20–30% of mTBI patients with normal CT scan present brain changes in structural or functional magnetic resonance imaging (MRI) (Shenton et al., 2012) (see more about this topic in section 5 of this chapter).

As noted previously, GCS is a measurement of severity for all TBI. In comparison with neurologically intact patients (GCS of 15), GCS scores of 13 and 14 have been associated with a higher risk of intracranial lesions, mortality and disability (Servadei, Teasdale, & Merry, 2002; Stiell et al., 2005; Teasdale et al., 2014). Due to this increased risk of complications, one supposition is that "only a score of 15 probably represents true mild TBI" (Alexander, 1995). However, there is no doubt that most patients obtain the maximum score, as studies report a GCS of 15 in around 80% of cases (Ratcliff et al., 2014; Yuh et al., 2013). LOC and PTA are other markers of increased severity, but their prevalence makes them irrelevant in most mTBI cases. The prevalence of LOC has been found to oscillate from 1% to 14.3%, and that of PTA between 2% and 29.7% (Carney et al., 2014). In addition, PTA is a much less reliable indicator in mTBI than in moderate and severe cases due to retrospective questioning and inherent difficulties in estimating short periods of time (that vary by minutes) under stress (King, 1997; Stuss et al., 1999). Neurological abnormalities, such as focal signs and seizures, occur only in a tiny fraction of mTBI cases.

The quest for stratification policies, together with the critiques of the adequacy of CT scanning in the mTBI population, has led to the identification of risk factors associated with a higher probability of intracranial lesions. As a result, the recommendation of performing CT scanning depends on these factors, which are considered of high or medium risk in combination with the presence of LOC and PTA (Jagoda et al., 2008). For adults, these include: age (≥ 65 or 60 years); GCS score less than 15; signs of open, depressed and basal skull fractures; two or more vomiting episodes; focal neurological deficits; coagulopathy; alcohol or drug intoxication; retrograde amnesia that extends for more than 30 minutes; and certain injury mechanisms (being a pedestrian struck by a vehicle, the ejected passenger of a motor vehicle, or falling from a height) (Ontario Neurotrauma Foundation, 2013a; Undén, Ingebrigtsen, Romner, & Scandinavian Neurotrauma Committee, 2013).

Notwithstanding, mTBI can occur in the absence of LOC, PTA and neurological signs and can produce no visible scarring on CT examinations. These clinical descriptors that emerged from severe TBI practice are negative in a considerable portion of mTBI cases and the picture they draw for mTBI patients is incomplete.

3.2. The concussion approach: clinical presentation

Leaving aside the mTBI–concussion debate, in response to the clinical need of various medical specialties, in 2011 the Brain Trauma Foundation and the Centers for Disease Control and Prevention founded the Concussion Definition Consortium, an evidence-based project (Carney et al., 2014). After conducting a systematic review of prevalent indicators, the task group did not put forward a nosological definition yet, but stated that:

> *"A concussion is a change in brain function that follows a force to the head, may be accompanied by temporary LOC and is identified in awake individuals with the use of measures of neurologic and cognitive dysfunction. (…) At this time, there are no known objective measures to identify the change in brain function called concussion. Consequently, observed signs, subjective reports and objective measures of neurologic and cognitive function that may be indicators of the underlying change in brain function are used to identify individuals with a high likelihood of having a concussion."*
>
> (Carney et al., 2014, p. s4)

The same task force identified several indicators of concussion, which they also delimitated in time in relation to the traumatic event. After a force to the head, an individual with a concussion can display the following:

1) *"Observed and documented disorientation or confusion immediately after the event,*
2) *Impaired balance, within 1 day after injury,*
3) *Slower reaction time, within 2 days after injury, and*
4) *Impaired verbal learning and memory within 2 days after injury".*

(Carney et al., 2014)

They define the first sign as "*the loss of one's sense of direction, position, or relationship with one's surroundings*" and the forth, concerning memory loss, as the impairment in the acquisition, retention, and retrieval of verbal material, in the memory of words and other abstractions involving language (idem, p. s7).

Note than none of these indicators is essential for a concussion diagnosis and that each of them can also be triggered by a variety of other causes unrelated to TBI.

3.3. Looking for common ground: clinically significant symptomatology

This lack of objective markers that can dictate a reliable diagnosis and guide personalized treatment is an unfortunate hallmark of the clinical management of concussion. As a result, one of the irreplaceable elements of mTBI assessment is subjective reporting. Patient-reported outcome tools are aimed at assessing the patients' subjective experience and their perception of change triggered by the traumatic incident. They include measurements of quality of life, health-related

quality of life, life satisfaction, and symptom checklists, among others. Self-reported symptoms are the most frequently reported of outcomes after mTBI (Cassidy et al., 2014)

The symptoms triggered by concussion are notoriously heterogenous and non-specific (Bigler, 2013; Ontario Neurotrauma Foundation, 2013b). In addition to confusion, imbalance, reduced processing speed and memory difficulties, concussion may translate into symptoms across a variety of functioning domains (**Table 3**).

Table 3. Common symptoms after mTBI and concussion–adapted from the clinical version of the Guidelines of Concussion/ Mild Traumatic Brain Injury and Persistent Symptoms, 2nd Edition (Ontario Neurotrauma Foundation, 2013)

Physical	**Cognitive**	**Emotional/ Behavioral**
Headache	Feeling "in a fog" or dazed	Depression
Balance problems	Feeling slowed down	Irritability
Dizziness	Difficulty concentrating	Anxiety
Nausea	Difficulty remembering	Emotional lability
Vomiting		* Drowsiness
Seeing stars or lights		
Blurred or double vision		
Sensitivity to light		
Sensitivity to noise		
Tinnitus		
* Fatigue		
* Difficulty falling asleep		
* Sleeping too much or less than usual		

* by some authors, these symptoms are included together in a different category, centered around a lack of energy. mTBI: mild traumatic brain injury.

In addition to the total number of symptoms, it can be relevant to pay attention to the symptom profile across clusters (Merritt, Meyer, & Arnett, 2015). Besides physical, cognitive and affective complaints, there are various symptoms that have in common a lack of energy or fatigue. Other classifications of concussion symptoms have been designed to guide the clinician in performing a complete assessment. For example, the COACH CV is an acronym indicative of seven putative concussion phenotypes, concerning cognitive problems, oculomotor dysfunction, affective disturbances, cervical spine disorders, headaches, and cardiovascular and vestibular anomalies (Craton, Ali, & Lenoski, 2017). For now, this approach has had limited echo in the clinical practice, despite its potential to prompt an early diagnosis of any potential dysfunction. A detailed review of the physiological, vestibulo-ocular and cervicogenic post-traumatic disorders is besides the scope of this thesis, but these syndromes have been gaining attention in the clinical management of concussion (Ellis, Leddy, & Willer, 2015, 2016).

In addition to looking for a reliable interpretation of self-reported symptoms, the quest to find objective measures of neurologic and cognitive dysfunction is ardent. These tools are generally performance-based standardized instruments that are considered more reliable in identifying acute effects of injury. The objective indicators of concussion are factors that weigh heavily in the decision to dictate the suspension of activity or specific treatments. Consequently, self-reported checklists are increasingly being accompanied by brief neuropsychological tests and neurological measures, especially for balance and motor coordination (Alla, Sullivan, Hale, & McCrory, 2009). In SRC, neuropsychological examination is considered one of the most relevant assessment tools (Zemek et al., 2016) and has been even deemed the cornerstone of SRC management (McCrory et al., 2017, 2013). In ED samples as well, cognitive instruments have been proved sensitive to changes after mTBI (De Monte, Geffen, May, & McFarland, 2010; Shores et al., 2008). Several neurocognitive protocols have been designed for SRC sideline evaluation, as Standardized Assessment of Concussion (SAC) and Sport Concussion Assessment Tool (SCAT), Concussion Vital Signs and ImPACT (Immediate Postconcussion and Cognitive Testing Test Battery) (McCrea, Asken, & Nelson, 2017). Traditional neuropsychological tools and computerized tests alike have showed that following mTBI and concussion, verbal learning and memory, information processing speed, response time and executive functioning can be altered (Carney et al., 2014; Rabinowitz & Levin, 2014).

4. Outcome and persistent post-concussion symptoms

There is little debate than abnormal physiological changes are the main cause of the initial symptoms of a concussion, but the disagreement regarding the natural trajectory following mTBI and the etiology of its long-term outcome has been substantial. The controversies about what constitutes poor outcome, reliable change indices and unbiased interpretation of results have divided the neurotrauma community.

4.1. Natural and delayed recovery

To begin with, the follow-up assessment following concussion is based on symptoms and mirrors the initial evaluation performed during the early presentation of such cases. It is generally reported that approximately 30% of patients do not fully recover within three months of the trauma and display unresolved symptoms in what was known as PCS (Williams, Potter, & Ryland, 2010).

A standard definition of PCS does not exist, and the analogous concepts include post-concussion(al) disorder and persistent post-concussion symptoms (PPCS). The latest version of the diagnostic and statistical manual of mental disorders, DSM-V (American Psychiatric Association, 2013), has replaced PCS with the concept of "mild or moderate neurocognitive

disorder due to TBI". All these descriptors refer to a constellation of symptoms that manifest after an mTBI or concussion and do not recover in a reasonable period of time. Most importantly, there is no consensus on the time window beyond which the recovery of concussion symptoms is considered pathological (Rose, Fischer, & Heyer, 2015). Based on sports-related concussion (SRC) studies, most athletes completely recover in the first 7–10 days following insult (McCrory et al., 2017). Therefore, some experts consider that any recovery that takes more than 10 days is worrisome and label those symptoms as persistent. Recovery after SRC proceeds in a sequential manner: usually balance problems resolve within 3 to 5 days, cognitive functioning reaches baseline levels within 5 to 7 days and self-reported symptoms gradually resolve by day 7 (McCrea et al., 2003). Other studies have also showed a progressive physiological and functional recovery following SRC (Kamins et al., 2017) and mTBI (Stuss et al., 1999). As expected, different modalities of measuring physiological change after SRC reflect different time courses of altered neurobiology. In terms of recovery of PTA, Stuss et al. have shown that the ability to recall three words freely after a 24-hour delay (what they considered "return to continuous memory") was recovered last, later than normal performance on the orientation and simple attentional task. As expected in terms of memory, the normal recognition of learned material was recovered before the ability to retrieve it. Put together, it appears that although it is not possible to identify a unitary window of physiological dysfunction following concussion, it is significantly lengthier than the duration required for clinical recovery. If clinical restoration can be achieved while physiological recovery is still ongoing, return-to-play/activity policies should be gradual and integrate a protective approach of a vulnerable brain to a secondary insult.

4.2. The injury, the brain and the person

Rowson et al. presented data on 25 concussions in the context of recording a range of biomechanical parameters from the helmets of 319 college football players and reported that concussive impacts did not stand out relative to impacts that did not result in injury. However, the concussive impacts were among the most severe for each individual player. They concluded that tolerance to head acceleration might be an individual characteristic, meaning similar biomechanical inputs can produce different injury manifestations (Rowson et al., 2017). This hypothesis of a variable tolerability to physiologic injury resembles the concept of brain reserve, as it seems to highlight individual features that modulate the response to a traumatic "dose" (Oldenburg, Lundin, Edman, Nygren-de Boussard, & Bartfai, 2016).

In fact, injury characteristics are just some of the factors that have been hypothesized to influence the presentation of PPCS. Some of the other variables that potentially play a role in triggering or maintaining the display of PPCS are presented together in **Figure2**.

Many characteristics have been associated with PPCS (Iverson et al., 2017; Quinn, Mayer, Master, & Fann, 2018; Tator et al., 2016). Genetic variation is involved extensively in the vulnerability for PPCS: some alleles increase the individual risk for mTBI, while others are

associated to the extent of injury or to various post-injury characteristics. Genetic factors modify the risk of PPCS in three different ways: by means of the role they play in the general preinjury state, in the host's initial response to insult and in the repair and plasticity processes (McAllister, 2015) (see more in **section 6.4.**).

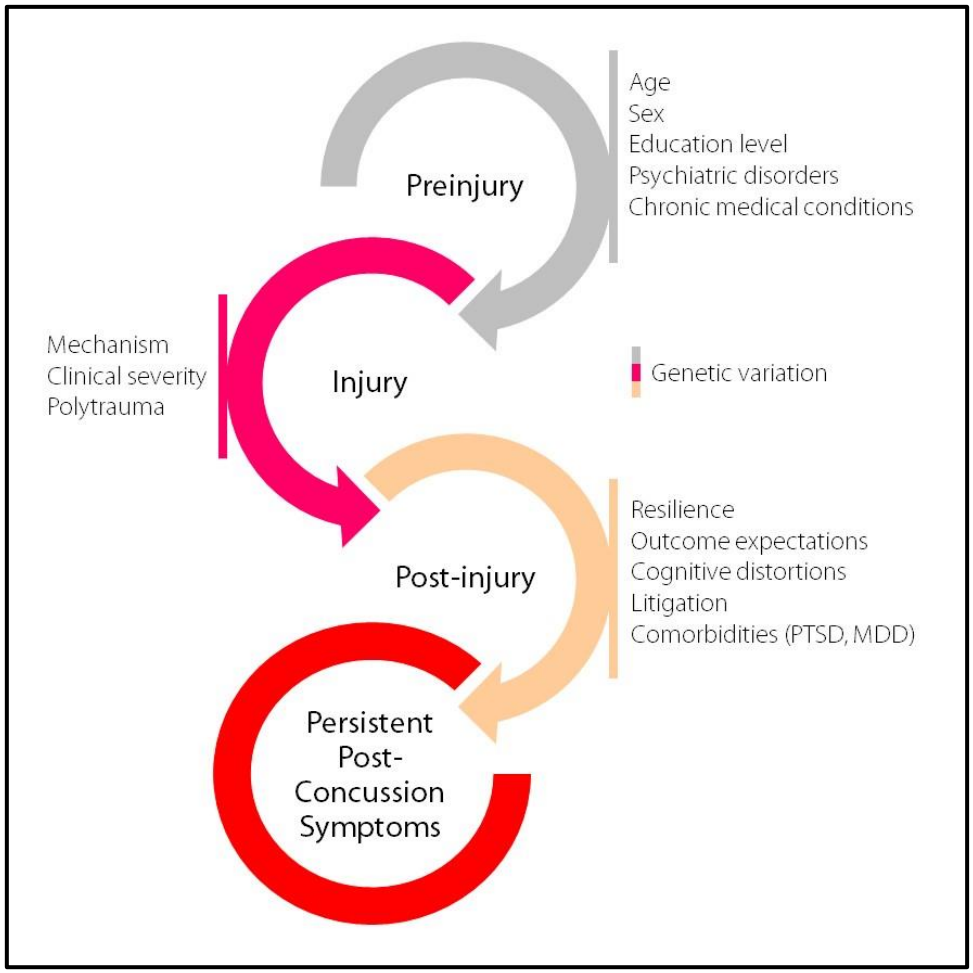

Fig. 2. Potential predictors for Persistent Post-Concussion Symptoms (PPCS). Among preinjury factors, adolescents and elderly patients, females and people with lower level of education have been associated with a higher risk. Chronic medical conditions and psychiatric history have been seen to increase the rate of PPCS. In addition to indicators of clinical severity, the mechanism of injury is among the most well studied peri-injury characteristics in relation to PCSS. The superposition of other syndromes such as post-traumatic stress disorder (PTSD), major depression disorder (MDD), migraines and other post-injury factors as litigation shape the post-concussion recovery and are associated with a poorer outcome. See text for references. mTBI: mild traumatic brain injury.

Amid demographic characteristics, age, sex, education level and occupational status are well studied, and most of the results point to females, pre-injury unemployment and an early end of the formal education process as risk factors (Ponsford et al., 2012; Yue et al., 2018). Traditionally, children have been considered less threatened by poor outcome in comparison with adults, which in turn were less exposed than the elderly (Anderson, Spencer-Smith, & Wood, 2011; King, 2014). Recently, being a teenager at the time of injury has been advanced as a stronger vulnerability than being of a younger age (Zemek et al., 2016). Not surprisingly, sustaining polytrauma and being the victim of assault are factors associated with worse outcome.

The indicators of clinical severity that have been presented in detail before, i.e. lower GCS, longer LOC or PTA and neurological signs, have naturally been studied in relation with a poorer prognosis. Other biological factors such as DAI, altered neurometabolism or cerebral blood flow are under scrutiny for their role in the development of PPCS (Chai et al., 2017; Helmich et al., 2015). For example, neuroimaging abnormalities are also markers of clinical severity. One proposed criterion advocates that the presence of ≥ 1 contusion or ≥ 4 shear foci visible on MRI significantly increases the risk for PPCS (Yuh et al., 2013). However, for decades, the inability to connect the post-concussion symptoms to objective brain injury evidence reinforced the hypothesis that what was causing the display of symptoms was a combination of psychological factors (personality traits, preinjury anxiety or depression) and contextual triggers (litigation, incentives for malingering) (Lees-Haley, Green, Rohling, Fox, & Allen, 2003).

Preinjury mental health, in general, but especially depression and anxiety disorders have been seen to increase the rate of PPCS (Scheenen et al., 2017). The same has been described for PTSD –which can be related or not to the same traumatic incident. Data on attention deficit with/without hyperactivity disorder, learning disabilities and alcohol/drugs use disorder seem to indicate similar roles, although with a minor impact (Iverson et al., 2017). Likewise, chronic medical conditions affecting general health status and a history of migraine and other TBI, including concussion, seem to worsen the outcome (Ponsford et al., 2012; Yuh et al., 2013). Some of the most important factors involved in the chronification of post-concussion symptoms or in delaying their resolution are thought to be post-injury. Resilience describes the individual ability to recover from adversity and implies an adaptive response to the post-concussion manifestations and the stress associated with a traumatic occurrence, treatment, suspension of activity, etc., if they take place. Resilient individuals have been shown to be less at risk for developing long-term symptoms (Losoi et al., 2014). Likewise, people with negative expectations about their recovery are supposedly affected by the nocebo effect, in the same way that some participants in clinical trials report side effects that they were informed could be associated with the treatment despite receiving only the placebo drug (Vanderploeg, Belanger, & Kaufmann, 2014). Another cognitive interference has been described as “the good old days” bias and designates an enhanced perception of loss that appears after mTBI, due to an overvaluation of preinjury levels. It has been shown that cohorts with mTBI report they were suffering from less concussion-like symptoms before the insult than the average person, and this inaccurate recollection of their premorbid condition magnifies their

perception of sequelae (Iverson, Lange, Brooks, & Rennison, 2010; Yang et al., 2014). Litigation is one of the most frequently mentioned factors that affect the mTBI outcome assessment, directly, by secondary benefits that arise with more severe symptomatology like monetary compensations or injury leave, and indirectly, as forensic examinations and in some cases a trial are additional stressors than can unequivocally delay recovery (Belanger, Curtiss, Demery, Lebowitz, & Vanderploeg, 2005; Lange, Iverson, & Rose, 2010). Like genetic variations, it is worth noticing that other factors like depression, chronic illness or personality traits can alter the course of the recovery by preconditioning the preinjury state and by the role they play after the time of the injury.

There are attempts to clarify this issue with novel methodologies, and a recent illustrative example was settled with pain as a post-concussion complaint. Post-traumatic pain and headache are frequent symptoms reported following concussion and mTBI. Kuperman et al. assessed a cohort of 100 patients in the first 72h following mTBI and found that clinical measures of head and neck pain correlated with multiple variables of the pronociceptive psychophysic response but were independent of most psychological measures (anxiety, depression, pain sensitivity). They concluded that the hypersensitivity and hyperalgesia involved in the early presentation of mTBI have a mostly organic basis, "free of mental influences" (Kuperman et al., 2018). For a deeper change of understanding of the PPCS, this approach should be replicated in other cohorts and extended to other symptoms.

The literature on PPCS has produced contrasting results, with not a single factor of the ones presented above having only confirmatory findings regarding its effect. In some cases, the balance appears tilted and, for example, the most consistent predictor of slower recovery is considered the severity of the acute and subacute concussion symptoms (Cassidy et al., 2014; Iverson et al., 2017; McCrory et al., 2017). In addition, it is important to keep in mind that in multivariable analyses of PPCS, many univariate findings lose significance. One striking example was given by Zemek et al., who out of 47 independent predictors for PPCS found that only 9 maintained significance in a multivariate model (Zemek et al., 2016). For clinicians, the need to understand the complex entanglement of factors related to concussion symptom reporting is crucial, as it is linked to the narrative of symptom attribution, treatment intensity, opportunity of follow-up or referral and, ultimately, to patients achieving a complete recovery.

4.3. Between the miserable minority and the fortunate few

The percentage of people with PPCS following mTBI varies remarkably in the literature, between 5% (Iverson, 2005) and 82% (McMahon et al., 2014). A discrepancy of this sort cannot be overlooked. Historically, there is a frequently cited prospective study from the 70s that reported at least one symptom in 14.5% of the cases one year after concussion, although less than 5% presented more than three symptoms (Rutherford, Merrett, & McDonald, 1979). Rooted in this study, for decades to come, 15–20% was considered the de facto percentage of concussion cases with poor outcome, ignoring limitations in that estimate. Based on these figures, only a minority of cases

would not recover in time and the term "miserable minority" was used to define them. For the lower end of the spectrum of PPCS incidence, Iverson argued that no study should report PPCS on the presence of singular symptoms. In the context of underreported mTBI cases and given the higher attrition of asymptomatic individuals, Iverson reasoned that the estimate of "true" PPCS is less than 5% of all concussions. Nearly a decade later, a multicentric study that prospectively examined 375 mTBI patients reported alarming data on chronic post-concussion symptomatology and loss of life satisfaction and global functioning. Both at 6 and 12 months after mTBI, 82% of the patients reported at least one symptom and more than 40% of cases had impoverished life satisfaction. At 12 months after mTBI, 22.4% still displayed some kind of functional disability, i.e. had a score below 7 in the extended Glasgow Outcome Scale (McMahon et al., 2014). In studies like this, the percentage of cases showing a complete recovery is practically inversed to what previously expected and it could be interpreted that only some "fortunate few" recover completely and timely. Clearly, conservative cut-off scores will produce lower PCSS estimates, but in favor of reporting cases with one symptomatic complaint lies the acknowledgement that currently there is no marker than can reliably exclude the possibility that any particular symptom is a consequence of mTBI. Until that becomes a possibility, researchers and clinicians will continue to oscillate between the type I and II errors in PPCS diagnostic, with some arguing that ignoring cases with few or singular symptoms is a form of mistreatment.

4.4. Facts and controversies in mTBI neuropsychology

The hypothesis that mTBI and concussion can produce clinically relevant impairment is linked to the thorny conversation around adequate outcome assessment. In addition to self-reported symptomatology that informs PPCS, outcome in individuals with mTBI is commonly established by assessing general disability, cognitive status, psychological health and quality of life (Shukla, Devi, & Agrawal, 2011). To begin with, clinicians and researchers agree that the assessment of general functional status, as measured by Glasgow Outcome Scale -extended (GOSe, Wilson, Pettigrew, & Teasdale, 1998), is not able to capture the heterogeneity of post-concussion repercussions. Just as the initial mTBI severity can be underestimated with GCS, the interpretation of mTBI outcome based on GOSe is biased by a ceiling effect and therefore many studies that report no general functional loss following mTBI can ignore persistent problems. Furthermore, to objectively inform on cognitive status is the aim of neuropsychological testing. Many studies have reported at months and even years after mTBI alterations in memory, processing speed, attention and executive functions (Barker-Collo et al., 2015; Dikmen, Machamer, & Temkin, 2003, 2016; Hanten et al., 2013; Heitger et al., 2006; Mangels, Craik, Levine, Schwartz, & Stuss, 2002; Nygren de Boussard et al., 2005; Ponsford et al., 2011; Rimel et al., 1981). More recent investigations have also depicted that years after an mTBI, some individuals present social cognition difficulties which are not explained by classic cognitive impairment (Theadom et al., 2019). However, the interpretation and clinical significance of these results was never straightforward.

The neuropsychology of mTBI has revolved for decades around two issues: the etiology of cognitive PPCS and inadequate methodological approaches. Mirroring the debate on PPCS in general, neuropsychology experts have been conflicted on the weight that brain injury has in explaining cognitive deficits, especially a long time after the traumatic event. On one side, as exposed previously, the early manifestations of a concussion event could be due to a purely physiologic disruption that, by existing neuropathological data, cannot trigger dysfunctions as the ones reported by patients or observed in neuropsychological evaluations months and years later. In addition, an amalgamation of psychological traits, mental health preconditions and comorbidities or plain malingering could easily produce the symptomatic profile of the "miserable minority". One of the most compelling arguments for discarding the explanatory function of brain lesion is the modest and inconclusive outcome difference found in multiple studies between cases with and without neuroimaging abnormalities. This is true not only for CT-visible injuries, but also for MRI markers (Hellstrøm et al., 2017; McCauley, Boake, Levin, Contant, & Song, 2001). That is to say that, in some series, there are individuals who recover completely following an mTBI with objective brain injury just as there are individuals who displays PCSS in the absence of evidence for lesions. However counterintuitive, the hypothesis that individuals who present with neuroimaging abnormalities following mTBI do not necessarily have a worse prognosis that those with normal CT/MRI findings cannot be rejected (more about the relevance of MRI investigations in mTBI clinical practice, in the following section of this chapter).

On the other side, there are historical considerations, neuropathological and neuropsychological data that accrue to a fundamental organic etiology of the cognitive deficits (and other symptoms) following mTBI and concussion. Historically, the psychogenic explanation has always been used to minimize the importance of symptoms and even discredit individuals who suffered from "neurosis", "diseases of the mind" and "weakness of will" until the technological advances produced compelling evidence of brain dysfunction in depression, schizophrenia, addictions, etc. Results from animal models and in vitro studies describe a neurometabolic cascade of concussion (Giza & Hovda, 2001, 2014). This neuropathological narrative links the concussion neurobiology with its early clinical manifestations and supports various judicious hypotheses regarding cellular processes that may underpin long-term impairment (Bigler & Maxwell, 2012). Using laboratory simulations of game impacts and highly detailed anatomical brain reconstructions, advanced biomechanical analyses applying finite elements models proved that, at least in SRC, the deformative strains are the highest in the midbrain, corpus callosum and fornix (Viano et al., 2005). In that study, loss of consciousness and dizziness correlated with early strain in the orbital-frontal cortex and temporal lobe, and memory impairment and other cognition problems correlated with late strain in the fornix and midbrain. This kind of modelling allows for putting forth explanatory hypotheses for most post-concussion symptoms: distortion of the upper brainstem and reticular activating system for alteration of consciousness; disrupted hippocampal-diencephalon-cingulate network, especially affecting the fornix, for mnesic deficits; medial temporal lobe and basal forebrain for emotional regulation; hormonal dysregulation due to hypothalamic-pituitary strain for fatigue and extensive stretching of the internal carotid, dura and

cerebral vasculature with diffuse irritation for headache (Bigler, 2008). An immense neurochemical and neuroimaging armamentarium has repeatedly exhibited post-traumatic changes following concussion and mTBI, and reasonably connected them to clinical manifestations (Bigler & Maxwell, 2012; Shaw, 2002; Shenton et al., 2012; Zetterberg & Blennow, 2016). Consequently, without disregarding current knowledge gaps, some experts consider that persistent neuropsychological deficits should be first and foremost attributed to the (mild) traumatic brain injury (Bigler, 2003).

Various neuropsychological studies, including meta-analysis and systematic reviews, have found no differences in long-term cognitive functioning between mTBI and control groups or such a small effect that is was interpreted as clinically insignificant (Frencham, Fox, & Maybery, 2005; Larrabee, Binder, Rohling, & Ploetz, 2013; Rohling et al., 2011; Rohling, Larrabee, & Millis, 2012; Schretlen & Shapiro, 2003). In response, Prof. Erin Bigler leaded a community of researchers that argued the meta-analytical approach is not adequate for the study of cognitive deficits following mTBI (Bigler et al., 2013; Iverson, 2010; Pertab, James, & Bigler, 2009). A meta-analysis could obscure clinically relevant information. They exposed the possibility of a bimodal distribution of mTBI outcome that remains unskewed despite nesting a relatively small sub-group with residual deficits, which remains masked under a meta-analytical approach. Nonetheless, consensus seems to have been reached regarding the small, rarely moderate, effect size that can be observed with conventional neuropsychological testing. A systematic review of 11 meta-analysis studies produced overall effect sizes between 0.07 and 0.61 for concussion in clinical samples, with some larger estimates for SRC (0.40–0.81) (Karr, Areshenkoff, & Garcia-Barrera, 2014). In 2005, Belanger et al. published what is considered one of the highest quality meta-analysis conducted to date and reported that in unselected or prospective samples the effect that mTBI has on cognitive status is not noticeable at 3 months or later ($d = 0.04$) (Belanger et al., 2005). Even the researchers who oppose meta-analytical approaches agree most individuals who sustain a mTBI recover and, concerning the ones that do not recover speedily enough, "from a clinical perspective these are negligible to minimal differences for the clinical neuropsychologist to detect with traditional neuropsychological measures in the individual with mTBI" (Bigler, 2013, p. 186). Taking into consideration that chronic impairment after TBI is estimated at 0.5 standard deviation below the average score, it would be unrealistic to expect more prominent deficits in the mild subgroup (idem). More recently, the International Collaboration on MTBI Prognosis (ICoMP) conducted the most extensive and rigorous systematic review up to date of studies published between 2001 and 2012, and presented consistent findings that mTBI is associated with neuropsychological deficits between 48 hours and 2 weeks after injury (Carroll et al., 2014). ICoMp also concluded that there is some evidence that cognitive recovery can be protracted for more than 3 months, although was not able to confidently advance more on the specific time required for recovery or specific persistent symptoms.

The synthesis of the best evidence on mTBI neuropsychological outcome is hampered by the variability of assessment tools. A systematic review of studies published between 2000 and 2012

reported that more than 700 unique instruments were used to assess outcome following TBI in adults, out of which 370 were for mental functions alone (Tate, Godbee, & Sigmundsdottir, 2013). Although neuropsychologists typically administer multiple psychodiagnostic tools, their focus is on cognition. The development of a reliable cognition endpoint in TBI research has been considered a priority, but the call remains unanswered, especially when dealing with mTBI population where the expected deficits are subtle (Bodien et al., 2018; Silverberg et al., 2017; Wilde et al., 2010). The variability of the sensitivity of cognitive tests to mTBI deficits between tests and between test batteries has been abundantly acknowledged (Bigler, 2013; Cicerone & Azulay, 2002; Karr et al., 2014; Levin et al., 2013; Pertab et al., 2009; Ruff, 2011). In addition, different tests are used to analyze distinct cognitive domains, such as attention, speed processing, memory, executive functioning, visuospatial processing, language, etc. Many instruments tap various functions and the composition of cognitive indexes sometimes follows different paradigms of cognitive hierarchy. Not surprisingly, the review literature on mTBI outcome found that early effect sizes and recovery rates vary between different cognitive domains.

For example, verbal fluency is a test frequently used in both neurological and neuropsychological examinations. The verbal fluency test involves a simple procedure of oral word generation in a controlled manner (i.e. in a specified period of time and generally restricted by a semantic or phonetic category), and it requires both verbal ability and executive control (Aita et al., 2018; Whiteside et al., 2016). Executive functions are heterogenous higher-order cognitive functions dependent on frontal systems, including shifting of mental sets, monitoring and updating of working memory representations, and inhibition of established responses (Miyake et al., 2000). Verbal fluency has been considered at times an independent function and at times a subcomponent of executive skills. What can seem an arid categorization problem has deep implications for data interpretation. In 2005, Belanger et al. published a meta-analysis on cognitive outcome following mTBI where fluency was the cognitive domain with the highest effect size when all results were collapsed across time since injury (d = 0.77) and the second highest effect size at less than 3 months following mTBI, after delayed memory (d = 0.89 and 1.03, respectively) (p. 219). In comparison, the smallest overall effects were reported for motor and executive measures (Belanger et al., 2005). In a meta-analysis dedicated to SRC longitudinal outcome conducted by two of the same authors, only one study of concussion included a test of verbal fluency, so the effect of fluency could not be analyzed separately. After computing verbal fluency together with variables of mental shifting, inhibition, abstract categorization and novel problem solving, executive functioning and attention were the only two domains that did not exhibit statistically significant deficits in the first week after SRC (Belanger & Vanderploeg, 2005). At the same time, in a smaller group of studies tackling cognitive outcome in athletes exposed to repetitive SRC, executive functioning was found to be one of the most vulnerable cognitive domains, displaying a moderate effect in comparison with control groups (d = 0.54). These ambiguous results have not been clarified by posterior studies, as nearly a decade later Karr et al. concluded that despite appearing most sensitive to multiple mTBI, the magnitude of the effects on executive functions remains unclear, ranging between -0.11 and 0.72 (Karr et al., 2014). These results highlight the ongoing

poor understanding on executive dysfunctions following mTBI, a topic particularly worrisome as it has implications beyond the characterization of mTBI outcome into general mTBI management, through its involvement in the dysregulation of self-monitoring - which is essential for reliable self-reported outcomes.

5. Diffuse axonal injury and the role of magnetic resonance imaging

It would appear that the debate regarding genuine, persistent and etiologically-consistent sequelae following mTBI and the lack of conceptual and methodological consensus has barely changed in over 30 years and that concussion research is at a standstill. If anything, the tremendous volume of recent publications, despite unavoidably bringing some contrasting results (that are partly implicit to scientific replication), proves a dramatic shift in the awareness of the repercussions of concussion and a sense of urgency for improving the understanding of its neuropathology. Without a doubt, advanced neuroimaging is the field that has generated knowledge with the highest impact in the mTBI research community.

Despite being the imaging modality of choice in acute mTBI, CT scanning has notorious limitations in identifying TBI lesions. Some of them have been known for decades (Gentry, Godersky, Thompson, & Dunn, 1988). CT is not a useful tool in describing subtle non-hemorrhagic traumatic lesions (Sharp & Ham, 2011). In 1994, Robert L. Mittl et al. put forth a pioneer work in which up to 30% from a small series with mTBI cases presented diffuse axonal injury on MRI despite normal CT findings (Mittl et al., 1994). This was considered a promising technique for identifying objective brain injury in cases where up to that moment the psychogenic etiology was compelling, and a powerful argument for improving mTBI management. Notwithstanding, the aim was not to replace CT with MRI as the primary exploration in head injury and will probably not be so for a long time to come, primarily due to limited availability and limited advantages in early diagnosis despite elevated costs. Currently, the use of MRI is aimed to identify the pathophysiological foundation of neurotraumatic lesions in concussion and, foremost, to achieve a better understanding of the outcome of these patients.

This section focuses on two concepts that are most relevant for the neuropathological characterization of mTBI through MRI: DAI and hemosiderin. There are many other putative neuroimaging biomarkers of interest for mTBI/concussion. For a comprehensive review of MRI findings, see Bigler, 2013, 2017; Gardner, Iverson, & Stanwell, 2014; Shenton et al., 2012; Wu et al., 2016; Yuh, Hawryluk, & Manley, 2014.

5.1. Axonal injury in concussion

DAI, sometimes referred to as traumatic axonal injury, is a process where mechanical forces induce stretching, torsion and shearing in axons and capillaries. In addition to primary axotomy, the transitory mechanical strain affecting the axon can progressively lead to axonal swelling, secondary axonal disconnection, retraction balls and Wallerian degeneration in the distal portion of the axon, spanning over various hours and weeks. Most frequently, DAI affects the subcortical white matter, corpus callosum and the midbrain and is a consistently strong predictor for functional outcome and cognition following TBI (Bigler & Maxwell, 2012; Junqué, 2008; Kraus et al., 2007; van Eijck, Schoonman, van der Naalt, de Vries, & Roks, 2018). In the classic view, neuropsychological alterations secondary to moderate or severe brain injury can be primarily explained by the localization of the visible brain lesions. (Silver, McAllister, & Arciniegas, 2009). As pure localizationism is outdated in neuropsychology, DAI is major candidate as explaining network dysfunction by altering the connectivity in systems that include multiple cortical and subcortical structures (Palacios et al., 2011). In addition, DAI is not visible on conventional CT, therefore it may explain the presence of residual symptoms and cognitive alterations following mTBI in cases previously believed to have structurally intact brain.

In the light of recent interest in concussion, various fundamental works in TBI neuropathology gain new implications. Although in clinical studies DAI after mTBI has not been described until 1994, the theory that axonal injury could explain the clinical manifestations of concussion is not completely new. In 1956, Sabina Strich produced the first detailed histological and neuropathological description of DAI. At the time, she acknowledged limitations in her conclusions but stated the following: "It is impossible to say whether the nerve fiber damage is at any time reversible and what part, if any, it plays in the production of the signs of concussion, but the possibility that it may play a part should be borne in mind." (Strich, 1956, p. 184) In another histology work, Peerless and Rewcastle introduced the term *shear* in relation with brain injury and carried Strich's conclusion further by adding that concussion could involve damage to the axon to the extent that it could disconnect the neuron (Peerless & Rewcastle, 1967). These findings called into question the transitivity of the concussion physiological basis and the assumed complete spontaneous recovery. More recent studies have corroborated these speculations, by histopathologically identifying axonal injury in patients who have suffered a concussion but died shortly after due to other causes (Blumbergs et al., 1995).

Although DAI can be inferred through various MRI sequences, for example fluid attenuation inversion recovery (FLAIR) and apparent diffusion coefficient maps, the method of choice for its analysis is diffusion tensor imaging (DTI). Various methods of DTI analysis produce quantitative parameters that describe white matter integrity by assessing the directional measurement of water diffusion in fiber bundles. In mTBI, DTI is sensitive to abnormalities in microstructural damage of white matter at a group level. DTI studies have shown that the splenium of corpus callosum is especially vulnerable to DAI (Aoki, Inokuchi, Gunshin, Yahagi, & Suwa, 2012), as is the subcortical white matter from the frontal and temporal lobes, especially at the

interface of white and grey matter. In histopathological samples, the severity of DAI is based on the location of the lesions and it is graded according to the involvement of grey–white matter junctions only (Grade I, of the lowest severity), and, additionally, of the corpus callosum (Grade II) and of the rostral brainstem (Grade III) (Adams et al., 1989). This gradient of severity is also suitable to lesions visible on neuroimaging scans.

Distinct patterns of DTI metrics are shown between early and chronic examinations, and a loss of white matter integrity has been associated with both increased and decreased fractional anisotropy, which is one of the most used parameters. One meta-analysis advanced that increased fractional anisotropy correlates positively with cognitive impairment in the first two weeks after mTBI but negatively later (Eierud et al., 2014). One hypothesis is that in the early stage following mTBI damaged white matter is affected primarily by edema, while axonal disconnection appears later. However, the time-since-injury that best distinguishes between recovery phases is uncertain (Dodd, Epstein, Ling, & Mayer, 2014). In addition to this directional variation of results, follow-up comparative DTI studies depend greatly on the use of the same MRI scanner because routine postprocessing relies of each scanner's normative set. Consequently, the interpretation of individual data has limitations despite the publication of promising results presenting DTI's added value in outcome prognosis (Yuh, Cooper, et al., 2014).

5.2. Vascular injury in DAI

DTI focuses on axonal alterations involved in DAI, but diffuse vascular damage and hemorrhages also take place in the same process. Hemosiderin is a by-product of blood degradation and a marker of microhemorrhages visible on MRI, as it induces inhomogeneities in the magnetic field just as deoxyhemoglobin and methemoglobin. Among MRI sequences that reflect vascular dysfunctions following mTBI, routine protocols include FLAIR, T2* and more recently gradient-recalled-echo (GRE) T2*-weighted imaging. Data from a recent multicenter used early CT scanning and a protocol of MRI that included FLAIR, GRE T2* and T1-weighted imaging in a cohort of 135 adults with mTBI (Yuh et al., 2013). Out of 98 patients lacking CT evidence of head injury, 27 (28%) had abnormal MRI: 23 cases with hemorrhagic axonal injury, 3 patients with brain contusions, and 4 patients with extra-axial hematomas. This study identified that presenting 4 or more foci of hemorrhagic axonal injury on MRI was associated with poorer 3-months outcome, with multivariate odds ratios of 3.2 ($p = 0.03$), after adjusting for CT findings and demographic, clinical, and socioeconomic factors. However, susceptibility-weighted imaging (SWI) is a technique highly sensitive to hemosiderin, and it has been proven up to 6 times more efficient than GRE T2* sequences in detecting punctiform bleeding associated with DAI in mTBI (Haacke, Xu, Cheng, & Reichenbach, 2004; Tong et al., 2003). Up to date, the relationship between brain microbleeds and cognitive or functional mTBI outcome has only been investigated in a few studies. In a series with mixed-severity TBI, SWI was seen to identify greater volume lesion than

FLAIR, and exhibited microbleeds in up to a third of the cases that had showed no abnormalities on FLAIR (Spitz et al., 2013).

Hemosiderin and other iron-storage compounds induce hypointense signal in SWI imaging and in mTBI are suggestive of hemorrhagic lesions that accompany DAI, but it is worth mentioning that not all DAI lesions are hemorrhagic. Non-hemorrhagic DAI foci are not visible on SWI, but on diffusion-weighted, FLAIR or T2/T2* imaging. Non-hemorrhagic DAI is far less frequent. In a study with a cohort of single mTBI that presented LOC and PTA in all 36 cases, 18 patients (50%) had lesions visible on CT and MRI identified intraparenchymal abnormalities in an additional 9 cases (25%) (Lee et al., 2008). By using a protocol that combined T1-weighted images, FLAIR and GRE-T2*-weighted images, MRI exposed 17 cases of hemorrhagic DAI (eight on CT), 4 cases of non-hemorrhagic DAI (none on CT) and 21 cerebral contusions (13 on CT). Patients exhibited reduced working memory at 2 weeks, 1 month and 1 year after mTBI but, despite increased detection of brain lesions, MRI findings did not correlate with cognitive impairment in this cohort.

6. Blood biomarkers for mTBI and concussion

Addressing the need for improving the understanding of mTBI pathophysiology and the weaknesses in diagnostics and care management is long overdue. One promising approach uses biofluid markers of injury, with blood being the most studied one due to an increasing availability of analysis techniques for blood samples with excellent detection power and with a relative low cost. In addition, there is a relative well tolerability of the extraction process. By comparison, post-injury markers have higher levels in cerebrospinal fluid (CSF) than in other biofluids and their concentrations are not influenced by the blood-brain barrier (BBB). Despite providing increased chances of detection, CSF sampling is considered too invasive to be obtained both in the general population and in the TBI patients that do not require the placement of a ventriculoperitoneal shunt for intracranial pressure assessment, as in mTBI. Blood biomarkers (BMs) are proteins, protein breakdown products, metabolites, lipids, peptides and mRNA fragments, among others, than can be detected in blood in relation to brain injury and that could primarily be used for a better understanding of its physiopathology and monitoring recovery (Jeter et al., 2013; Zetterberg, Smith, & Blennow, 2013). Because the current incomplete explanation that links clinical manifestations, underlying brain changes and outcome in mTBI, the utility of BMs can be assessed in three distinct areas: detecting concussion/mTBI, predicting intracranial damage and predicting unfavorable outcome (Gan et al., 2019).

Currently, there are dozens of proteins and biological elements under scrutiny for their potential role in pinpointing brain injury in concussion. A complete review is beyond the scope of this work, but the reader is referred to Papa, Edwards, & Ramia, 2015; Papa, Ramia, Edwards,

Johnson, & Slobounov, 2014; Zetterberg & Blennow, 2016; Gan et al., 2019. This section will focus briefly on a theoretical model of the neuropathological biochemical process that takes place in concussion, will enumerate some of the most well studied BMs and will expose a rationale for the BM panel designed in this thesis. Lastly, it will review a series of genetic biomarkers for mTBI, considering that genetic markers, commonly identified in blood samples, are increasingly being used to understand susceptibility to traumatic insult and recovery following concussion.

6.1. The metabolic cascade of concussion

The model of the neurometabolic cascade of concussion is similar to the pathophysiology phenomena of more severe TBI, but it involves processes of lesser magnitude and duration. It has been established after the review of compelling results in experimental animal and in vitro studies, and several findings have been corroborated in human studies (Barkhoudarian, Hovda, & Giza, 2011; Giza & Hovda, 2001, 2014; Giza, Greco, & Prins, 2018).

At the neurochemical level, the first consequences of a biomechanical injury are an abrupt neuronal depolarization and indiscriminate release of neurotransmitters, especially excitatory amino acids like glutamate, which explains the excitotoxicity that is associated with neurotraumatic damage. The mechanoporation of the cellular membrane, which occurs directly as a transient disruption after the mechanical deformation, is accompanied by increased potassium efflux and sodium and calcium influx. This uncontrolled ionic imbalance rapidly triggers a cellular energy crisis, due to the depletion of adenosine triphosphate (ATP) by the sodium-potassium pumps that strive to restore cellular homeostasis. This also induces an acute period of hyperglycolisis, aimed at generating more ATP, and will be followed by a relatively long period of decreased cerebral glucose metabolism (7 to 10 days in adult animals and, estimated, in humans). Hyperglycolisis leads to the accumulation of lactate, triggering local acidosis and membrane damage, among others. Lactate can also be used as an energic substrate by neurons, but only under normal mitochondrial function. In concussion, the ionic disequilibrium is expected to resolve in a matter of hours, except for the increased levels of intracellular calcium that can persist for days. In response, the cell initiates the sequestration of calcium into the mitochondrial matrix, which leads to mitochondrial dysfunction and impaired oxidative metabolism. At the same time, this status of oxidative stress induces the degradation of the cellular membrane, through lipidic peroxidation. The mitochondrial dysregulations affect the energy supply of the axolemma and provoke a further increase of calcium intake. In some cases, this can activate the opening of the permeability transition pore and eventually lead to mitochondrial failure and cellular death.

Neurons, endothelium cells, astrocytes and their microstructural components are damaged in concussion, initially as a result of direct mechanical deformation. Axons are particularly sensitive to the stretching induced by rotational and acceleration–deceleration forces. Cytoskeletal damage, that encompasses phenomena such as microtubule misalignment and neurofilament compression, is accompanied by axonal dysfunction and deters neurotransmission. In more

extreme cases, the rupture of the axon takes place, which explains the presence of retraction ball–the histopathological hallmark of DAI–and the subsequent processes of Wallerian degeneration. Moreover, the fact that unmyelinated white matter fibers are especially vulnerable to cytoskeletal damage in comparison to myelinated ones makes the corpus callosum a location greatly susceptible to injury. It is important to note than in most cases axonal injury and even secondary axotomy do not invariably lead to neuronal death, as it was once believed, but to neurons with atrophied cellular bodies that are dysfunctional. Despite the long-term complex biochemical aftermath and secondary injuries, only a tiny fraction of neurons dies following a single concussion (Giza & Hovda, 2014).

The biochemical changes induced by mTBI must also take into consideration the traumatic cerebral vascular injury (TCVI) (Kenney et al., 2016). TCVI is not restricted to mechanically disrupted vasculature and microbleeds. In concussion, as well as in more severe types of TBI, it includes endothelium cell damage, altered cerebral blood flow, reduced cerebrovascular reactivity and impaired BBB (Johnson et al., 2018; Kenney et al., 2016; Werhane et al., 2017). The regulation of the brain blood compartment and its functional coupling with neuronal activity and glucose metabolism is achieved through a network of neurovascular units (NVUs), which are dynamic structures comprised of endothelial cells, astrocytes, pericytes and the adjacent neurons, among others (**Fig. 3**) (McConnell, Kersch, Woltjer, & Neuwelt, 2017; Nag, Kapadia, & Stewart, 2011). Given the entanglement between cerebral microvasculature and other parenchymal components, the NVU seems like an excellent target for studies of primary and secondary traumatic injuries.

Indicators of TCVI and NVU dysfunction have been observed following concussion in animal (Johnson et al., 2018; Tagge et al., 2018) as well as human studies (Bartnik-Olson et al., 2014; Meier et al., 2015; Tagge et al., 2018). In models of fluid percussion injury, there is an immediate reduction in blood flow of up to 50% that persists for days (Giza & Hovda, 2001). Until recently, BBB dysfunction was thought to be restricted to moderate or more severe injury, but experts are acknowledging it can occur following concussion too (Johnson et al., 2018; Kenney et al., 2016).

Another set of biochemical changes that take place following mTBI in both early and chronic stages is neuroinflammation, which involves a release of interleukins, cytokines and matrix metalloproteases. While it was widely described in more severe neurotrauma, neuroinflammation following concussion has been frequently neglected (Giza & Hovda, 2014; Patterson & Holahan, 2012). However, some researchers have considered its role in the biochemical cascade after concussion crucial to such an extent that they put forth the concept of "post-inflammatory brain syndrome" to replace PCS (Rathbone et al., 2015). In addition to the call of macrocytes and neutrophils to the injured areas due to their role in cytokine release, the brain has specific strategies for this and activates microglia and astrocytes, with the same purpose. S100 calcium-binding protein β (S100β), a glial-derived cytokine, and glial fibrillary acidic protein (GFAP), which is often used as a marker of astrocyte activation, are two of the most promising candidate BMs in concussion, as described below (Zetterberg & Blennow, 2016). It is worth noticing that

neuroinflammation appears to have both beneficial and detrimental effects; while a prolonged exposure to cytokines is eventually deleterious, neuroinflammation also has a potentially neuroprotective role and is involved in the clearance of debris, repair and regeneration following mTBI (Patterson & Holahan, 2012).

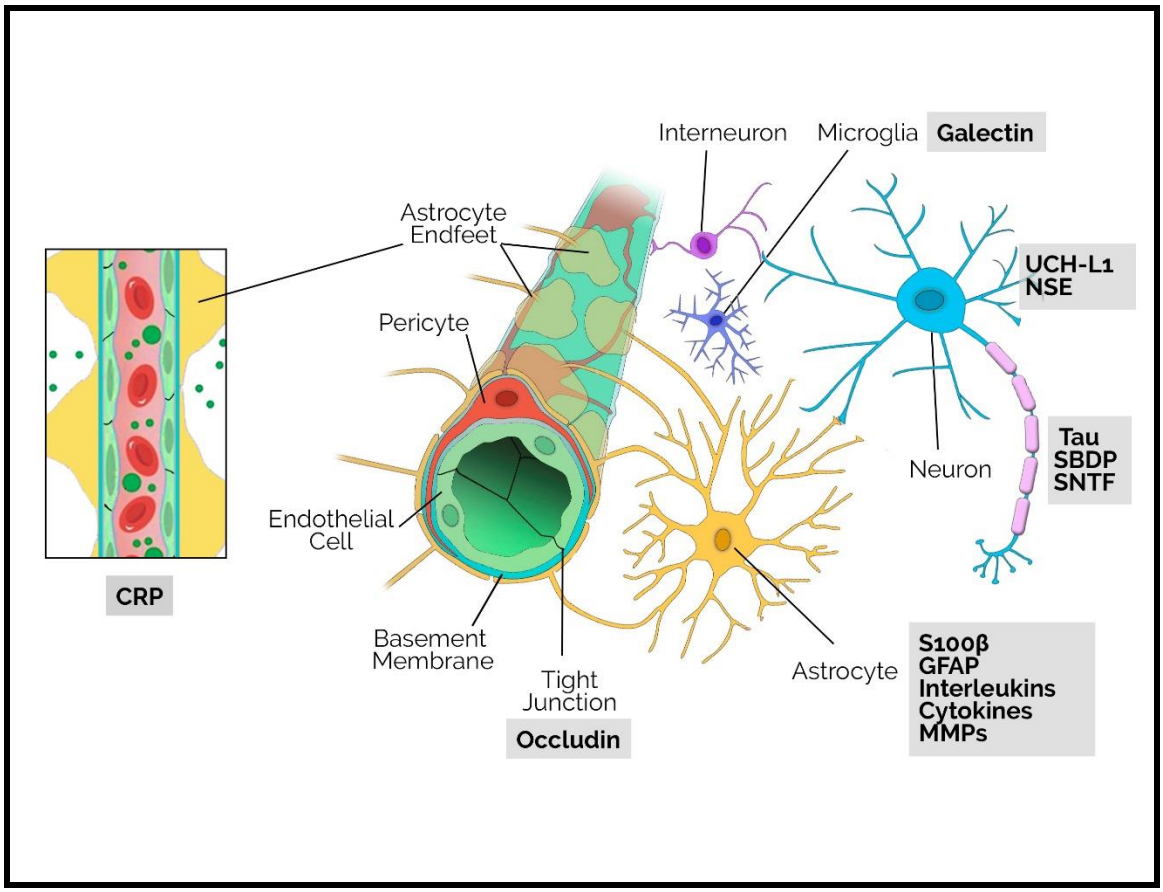

Fig. 3. The schematic structure of a neurovascular unit and the blood-brain barrier (BBB) (in the left rectangle). The capillaries contain a single layer of endothelial cells connected by tight junctions. Pericytes regulate the diameter of the capillaries and maintain the integrity of the BBB. Cerebral vessels receive inputs from astrocytes by their end-feet, as well as input from interneurons. The neuronal bodies are rich in UCH-L1 and NSE, which are markers of neuron injury. Tau, spectrin breakdown degradation products (SBDPs) and the calpain-derived αII-spectrin N-terminal fragment (SNTF) are indicative of axonal damage. Activated astrocytes release S100β, glial fibrillary acidic protein (GFAP), interleukins and other cytokines and matrix metalloproteinases (MMPs). Galectin is primarily expressed by microglia. Systemic inflammation markers, as C-reactive protein (CRP), could also play a role in the pathophysiology of mTBI (Adapted from Brown et al., 2019, under a Creative Commons Attribution CC-BY License)

As the reports on mTBI will continue to present levels of BMs in blood, it is important to interpret those results within the wider neuropathological frame: understand the fundamental biological function of the protein of interest and the implications of their altered levels in systemic blood, taking into consideration the origin of the protein, when it is detected in relation with injury (early detection could imply a release from injured cells and a delayed detection could reveal upregulation for repairing), etc. (Kawata et al., 2016) Notably, the presence of proteins from central nervous system in systemic blood stream can be explained by the alteration of the BBB. However, a more recent hypothesis focuses on the role played by the glymphatic system in the clearance of brain-derived proteins after injury. The glymphatic system relies on the exchange of abnormal proteins and metabolites between interstitial fluid and CSF and their further release into the cervical lymphatic system from where they reach peripheral circulation. Although further research is needed, in a murine model, Plog et al. reported that after blocking the glymphatic system, the levels of S100β, NSE and GFAP could not be detected in serum after experimental TBI (Plog et al., 2015).

6.2. Prominent candidate blood BMs

The use of blood BMs for concussion has been applied in two exemplar cases. First, the Scandinavian guidelines for the management of mTBI introduced a restriction for CT scanning in the absence of elevated S100β levels (Undén et al., 2013). The rationale for these guidelines was based on numerous studies showing that S100β is highly sensitive after TBI (Heidari, Asadollahi, Jamshidian, Abrishamchi, & Nouroozi, 2014; Thelin, Nelson, & Bellander, 2016). Second, in early 2018, the U.S. Food and Drug Administration approved a commercially-available kit that incorporated ubiquitin C-terminal hydrolase-L1 (UCH-L1) and GFAP to rule out the indication of CT scanning, if performed in the first 12 hours following mTBI. They based their decision on a multicenter prospective study of a cohort of nearly 2000 adults, in which the kit proved a 97.5% positive prediction value and a 99.6% negative predictive value for patients with intracranial lesions visible on CT scans (Bazarian et al., 2018). Although these cases set precedents for the modification of clinical mTBI policies by integrating reliable blood BMs, there is a considerable hiatus between the marketed aim of the kit, that is "to diagnose concussion", and the actual interpretation of the results, that is to exclude the indication of a CT scan. These tests have no informative value regarding the possibility of having a concussion with negative CT findings, nor about the possibility of presenting intracranial lesions visible on MRI and indeed nothing to the possibility of presenting PPCS. While the importance of reducing unnecessary radiation exposure from medical imaging should not be underestimated, currently the are no BMs and no "BM signature" (a specific combination of various BMs) with confirmed diagnostic nor prognostic accuracy for concussion and PPCS. In **table 4** are included the most relevant protein BMs for the study of concussion, based on either extensive investigation or very promising preliminary results in single cohorts (such as ghrelin, occludin, SNTF).

Table 4. Candidate protein BMs for enhancing the understanding of concussion physiopathology and its outcome (adapted from Gan et al., 2019)

Aim	Biomarker	Nº reports	Nº observations[1]	Pooled AUC
Detecting the presence of concussion	Panel: copeptin, galectin 3, MMP-9	1	55	0.968
	Panel: GFAP, UCH-L1	1	206	0.940
	occludin	1	55	0.836
	S100β	2	108	0.680
Identifying the need for CT scan	Panel: GFAP, UCH-L1	1	1947	0.986
	Panel: MMP-2, CRP, CKBB	1	110	0.964
	T-Tau/ P-Tau ratio	2	350	0.923
	d-dimer	2	93	0.890
	GFAP/GFAP-BDP	16	2040	0.831
	NSE	5	844	0.798
	S100β	30	8464	0.723
	CRP	1	92	0.714
	UCH-L1	5	3108	0.700
Predicting delayed recovery	A-Tau	1	56	0.870
	SNTF	2	73	0.863
	ghrelin	1	118	0.829
	neurofilament light	1	35	0.820
	UCH-L1	7	3158	0.787
	GFAP	17	1959	0.716
	S100β	24	2800	0.691
	NSE	6	543	0.685
	CRP	1	846	0.615

A-Tau: Tau-protein A; AUC: area under the receiver operating characteristic curve; CKBB: creatine kinase B type; CRP: C-reactive protein; GFAP: glial fibrillary acidic protein (breakdown products); MMP-2/-9: matrix metalloproteinase 2/9; NSE: gamma-specific enolase; P-Tau: hyperphosphorylated Tau; UCH-L1: ubiquitin C-terminal hydrolase; SNTF: calpain-derived αII-spectrin N-terminal fragment; T-Tau: total Tau.

[1]The number of observations is computed in the original paper of Gan et al., 2019 and represent the number of determinations per protein or protein panel included in the pooled AUC determination, not necessarily the same as the patient sample size of the studies.

S100β is one the most well-known studied BMs in TBI, as is gamma-specific enolase (previously called neuron-specific enolase, NSE). S100β is a protein that plays a role in the regulation of intracellular levels of calcium and is very sensible following glial injury although it is also expressed following extracranial fractures, whereas NSE is a glycolytic enzyme that arises in

response to neuronal injury. Although NSE is also present in erythrocytes and endocrine cells, it is considered specific to brain injury but poorly sensitive (Jeter et al., 2013). Both S100β and NSE have been seen to correlate with unfavorable outcome and complications following TBI, but various limitations to their use remain. In addition, in multivariable models, the predictive capabilities of NSE together with S100β have not surpassed those of S100β alone, which led to the questioning of the clinical utility of NSE (Thelin, Jeppsson, et al., 2016).

UCH-L1, one of the proteins in the BM kit approved by the U.S. Food and Drug Administration to aid in concussion assessment, is highly abundant in neurons and was previously used as a histological marker for neurons. GFAP, the second one, is expressed by astrocytes and is a brain-specific protein that is released following TBI. In moderate and severe TBI, the levels of GFAP in serum are predictive of mortality, poor outcome and other TBI complications (as increased intracranial pressure). Other examples include tau protein and products relative to alpha II spectrin (as SNTF), which is abundant in axonal structure (Dambinova et al., 2016; Siman et al., 2014; Zetterberg & Blennow, 2016). Ghrelin, a gastric peptide that plays a role in appetite upregulation, is thought to prevent disruption of the BBB and to diminish the inflammatory response by altering leukocyte recruitment after (Xu, Lv, Wang, Chen, & Qiu, 2014).

6.3. A novel protein biomarker panel

One of the aims of this thesis was to introduce a comprehensive BM panel to detect two neglected aspects of TBI outcome: the vascular functionality (Nag et al., 2011) and the neuroinflammatory response (Woodcock & Morganti-Kossmann, 2013). Selected BMs were chosen for analysis in addition to a reliable marker of glial damage, S100β.

The vascular endothelial growth factor (VEGF) is considered one of the most powerful factors involved in angiogenesis and modulation of endothelial permeability, dilation and proliferation (Yancopoulos et al., 2000) but VEGF also enhances neurogenesis and angiogenesis and reduces lesion volume after TBI (Thau-Zuchman, Shohami, Alexandrovich, & Leker, 2010). Like VEGF, angiopoietin 1 (Ang-1) is an essential protein for normal vascular development. It has been proposed that Ang-1 and angiopoietin 2 (Ang-2) are pro- and anti-angiogenic, but it has been shown that Ang-2 can be converted into a proangiogenic factor under VEGF-dependent modulation (Lobov, Brooks, & Lang, 2002). The von Willebrand Factor (vWF) is an endothelium-specific glycoprotein primarily involved in hemostasis in response to endothelial damage. Elevated vWF serum level is considered a marker of increased vascular permeability and it has been associated with unfavorable outcome after severe TBI (Ahmed, Cernak, Plantman, & Agoston, 2015; De Oliveira et al., 2007).

TBI is accompanied by a vigorous expression of inflammatory agents. Interleukin 1β (IL-1β) is one of the most studied cytokines in response to brain trauma, but its clinical relevance has not yet been established, as it is notoriously difficult to detect in humans (Woodcock & Morganti-

Kossmann, 2013). Caspase 1 (Casp-1) is the enzyme regulating the maturation of pro-interleukin 1b to its pro-inflammatory and active form. Leptin (Lep) has been traditionally described as a satiety factor, but it is also a pro-inflammatory adipokine (Ouchi, Parker, Lugus, & Walsh, 2011). Its involvement in regulating inflammatory response is multiple, as leptin mRNA expression and circulating leptin levels are regulated by various inflammatory agents, including IL-1, while leptin expression enhances the production of C-reactive protein (CRP), as well as tumor necrosis-alpha and interkeulin-6 (Lin, Huang, Wang, & Shen, 2012). In addition, leptin appears to mitigate neuronal death in both in vitro and in vivo models of human disease (Avraham et al., 2011; Signore, Zhang, Weng, Gao, & Chen, 2009). In a cohort of pediatric severe TBI cases, elevated acute plasma leptin levels were associated with increased mortality and unfavorable outcome (Lin et al., 2012). CRP is an acute-phase protein synthesized by the liver and has been described as a sensitive but nonspecific biomarker which levels increase in response to inflammation following infection, trauma, surgery and many more (Gabay & Kushner, 1999). Serum CPR values exhibited a robust increase after severe TBI (Hergenroeder et al., 2008). In a pilot mTBI study, Su et al. showed that elevated CRP baseline levels could predict persistent unfavorable outcomes (Su et al., 2014).

6.4. Genetic biomarkers in mTBI

A growing body of literature has been focusing on genetic factors that play a role in the pathophysiology of TBI, and their usefulness in explaining outcome variation (Jordan, 2007; McAllister, 2015). To begin with, preinjury functioning is undoubtedly influenced by genetic makeup of each individual. Subsequently, the heterogeneity of TBI presentation is partly explained by individual differences in the genetic modulation of inflammatory processes, vascular response to trauma and apoptosis. The long-term outcome following TBI, which is still notoriously difficult to predict in most cases and particularly in mTBI, is strongly influenced by plasticity and repairing processes, that are also under genetic regulation. More than two decades ago, the apolipoprotein (APOE) ε4 allele was shown to be an independent factor for an unfavorable outcome following severe TBI (Teasdale, Nicoll, Murray, & Fiddes, 1997). The results put forward by this and other association studies have been put into perspective by more recent studies showing that neurotrauma triggers genome-wide changes of transcription and methylation factors, and alters whole gene networks which are relevant to brain function (Meng et al., 2017).

In addition to genes that play a role in TBI pathophysiology, candidate genes for investigation into post-traumatic outcome are selected based on the hypotheses concerning cognitive and emotional functioning. Various polymorphisms that have been linked to general neuropsychological functioning have been studied in relation with the recovery following TBI. Results on a cohort of penetrating TBI support that the variant Met66- of the brain derived neurotrophic factor (BDNF) gene, which is highly expressed in the hippocampus, facilitates the recovery of executive functions after TBI, despite having associated with poorer episodic memory, working memory and hippocampal function in healthy population (Krueger et al., 2011).

Neurogenetics studies have shown that the Val158Met variant of the catechol-o-methyltransferase (COMT) and specific alleles of the dopamine receptor D2 (DRD2) gene are protective factors (Jordan, 2007). COMT encodes an enzyme involved in the degradation of catecholamines, such as dopamine. The allele which is less active (i.e. Met) is associated with a 30% reduction in enzymatic activity (Nielson et al., 2017), therefore leaving more dopamine in the synaptic space and involving an cognitive advantage. The ankyrin repeat and kinase domain containing 1 (ANKK1) gene regulates the synthesis of dopamine in the brain and it is located very closely to the DRD2 gene, up to the point where until recently the polymorphism Taq1 of the ANKK1 gene was believed to belong to the DRD2 promoter region. Taq1 and multiple allelic variants of single-nucleotide polymorphisms (SNPs) in the DRD2 have been associated with cognitive variation following TBI, in working memory, impulsivity, inhibition and memory (Bales, Wagner, Kline, & Dixon, 2009).

Together with various genes involved in striatal dopaminergic processing (ANKK1, COMT, DRD2), poly(ADP-ribose) polymerase-1 (PARP-1) was found to be related with brain posttraumatic outcome, especially after mTBI with negative CT findings (Nielson et al., 2017). PARP-1, encoded by the eponymous gene in the human chromosome 1, is a ubiquitously expressed enzyme that plays an important role in the cellular response to DNA damage and stress. PAPR-1 is also known as NAD^+ ADP-ribosyltransferase 1, as it uses NAD^+ as a substrate to add ADP-ribose to nuclear proteins. Up to 200 molecules of NAD+ can be used for the repairing of a single protein, so the overactivation of the poly-ADP-ribosylation (PAR) can lead to energy failure and cell death (Virág & Szabó, 2002). Two studies have focused on an indirect measurement of PARP-1 modified proteins after TBI, expressed in CSF, and showed that an increase in these levels is measurable in pediatric samples (Fink et al, 2008) and that they can be linked with outcome following TBI (Sarnaik, 2010). The increase in PAR-modified proteins in CSF after TBI may be due to increased PARP activation, decreased PAR degradation, or both.

In mTBI research, it is assumed that PPCS reflect a greater cognitive vulnerability, which can be conditioned partly by the genetic predisposition of individuals (Junqué, 2008). However, the role played by risk polymorphisms for the recovery following mTBI has not been understood in depth.

After reviewing the most important findings in the literature on mTBI and concussion, this thesis was based on the acknowledgement that there is an imperious need of improvement in the clinical management of these patients. It became apparent that there were several gaps in the current understanding concerning the presentation of patients with concussion, the suitable tools of early assessment in these cases and a lack of reliable predictor factors for their recovery.

The following studies, together with the analyses included in the supplementary material, were designed to fundamentally tackle these issues. In order to achieve that, we planned to recruit a cohort of otherwise healthy adults and conduct a longitudinal follow-up for up to 3 months following mTBI. First, clinical, neuropsychological, biochemical and neuroimaging investigations were planned for a comprehensive, multidimensional characterization of the potential brain lesions and the accompanying symptom profile. The goal was to broaden the insufficient hospital assessment protocol by combining (1) novel tools that could potentially be included in routine evaluations, (2) a suitable neuropsychological battery, (3) MRI examination (in addition to the clinically-indicated CT scan) and (4) an original panel of blood biomarkers.

One of the recurrent limitations of the prognosis models we reviewed was the use of suboptimal measures of injury severity and outcome. Clearly, it is difficult to rate the predictive power of biomarkers and other factors if the clinical measures they are being compared against lack adequate psychometric properties. Furthermore, the lack of specificity of the symptoms used to identify poor outcome following mTBI, i.e. PPCS, is a thorny issue. Therefore, we aimed at improving this by defining the clinical thresholds used for the identification of PPCS with a rigorous methodological approach. This study was designed in a cohort of healthy adults of similar characteristics with the mTBI group, but with no history of head injury. The analysis of the concussion-like symptoms exhibited by the healthy participants was intended to be performed together with a thorough clinical and neuroimaging characterization.

As described previously, a panel of blood BMs was designed for this thesis. Selected markers of endothelial damage, vascular dysfunction, neuroinflammation and glial injury were assessed in terms of their predictive potential for clinically significant PPCS. The identification of cases with delayed recovery was designed to use the results of the previous study, in addition to traditional criteria. This data is presented in the Supplementary Material, Section B.

Lastly, several genetic polymorphisms that are hypothetically associated with a differential risk of delayed recovery following mTBI and concussion were examined. The analysis conducted on genetic vulnerability in relation with the PCSS and cognitive profile is presented in the Supplementary Material, Section D.

II. Hypotheses and Objectives

This project was aimed at proving or refuting the following hypotheses:

H1. mTBI results in psychological, cognitive and behavioral sequela in a non-negligible percentage of patients, which are detected not only in the first days but even at least 3 months after the traumatic event, altering the social and work return of these patients.

H2. Case identification of poor recovery following mTBI can be improved by defining incomplete recovery with data-driven approaches that are based on the presence of concussion-like symptoms in the general population with no history of head trauma. Because outcome assessment in mTBI is flawed by lack of consensus between experts, the use of a robust empiric method of outcome classification should yield a more homogenous subgroup of mTBI patients with persistent post-concussion symptoms.

H3. mTBI objectively affects the brain at a structural and functional level. These cerebral lesions can be detected by means of advanced MRI and biochemical analyses of BMs sensitive to brain injury. Specifically, we expect mTBI patients to show higher concentrations of brain injury-specific BMs than the control group, and these concentrations to relate to post-concussion outcome.

H4. The mTBI sequela are conditioned by the patient´s genetic makeup. Being a carrier of specific alleles of the genes ApoE, ANKK1, BDNF, COMT and PARP-1 is associated with a worse cognitive recovery following a mTBI.

H5. Among the descriptors of the patient's early state and his or her premorbid characteristics there are reliable predictors for its cognitive and behavioral state three months following an mTBI.

The main aim of this project was to assess the prevalence of post-concussion symptoms and cognitive alterations during the early stage following an mTBI, their presence in the first 2 weeks and at 3 months post-mTBI and the relationship between the neuropsychological profile with clinical, neuroradiological and genetic factors, and specific blood biomarkers.

The specific objectives are:

O1.H1 Determine the prevalence of cognitive and behavioral sequela in a group of patients with mTBI, in the early stage (in the first 14 days) and 3 months (90 ± 14 days) later, and describe the longitudinal profile of mTBI neuropsychological recovery.

O2.H1 Determine the longitudinal profile of post-concussion symptoms in the mTBI group and compare distinct strategies for the identification of incomplete recovery using traditional cut-off points and novel data-driven normative scores.

O2.H2. Compare the concentrations of blood BMs between the patients with mTBI and a control group in order to objectively describe the severity of cerebral injury.

O3.H2. In a subgroup of cases, all with negative CT findings, identify the presence and extension of DAI lesions by using suitable MRI sequences.

O4.H3. Analyze the relationship between the selected genetic biomarkers and the neuropsychological profile of the participants.

O5.H4. Investigate into correlations between the cognitive-behavioral status at both assessment points, premorbid descriptors and the BM levels.

O6.H4. Identify very early factors with predictive value for the persistence of post-concussion symptoms at three months following an mTBI.

III. Participants and Methods

1. The mTBI group

Between April 2013 and April 2014, patients that attended the Neurotraumatology Unit of Vall dHebron University Hospital's emergency department were screened. To be included in the study, patients had to meet all the inclusion criteria and none of the exclusion criteria listed below.

Inclusion criteria:

1. age between 18 and 65 years,
2. being a fluent speaker of Catalan or Spanish,
3. having had mTBI with a GCS score of 14–15 in the 24 h prior to study inclusion,
4. having experienced concussion, identified by loss of consciousness lasting < 30 min (verified by a witness), vomiting, seizures, PTA lasting < 24 h, or intense post-concussive symptoms (**Table 5**),
5. normal neurological examination findings, and
6. normal brain CT findings.

Exclusion criteria:

1. previous head trauma requiring hospital care;
2. history of chronic substance abuse;
3. known psychiatric or neurological condition;
4. chronic systemic disease with potential cognitive effects (renal insufficiency or kidney failure, metabolic syndrome, etc.); and
5. polytrauma with an Injury Severity Scale score above 6.

Neurological examinations and initial interpretation of brain CT scans were performed by the on-call neurosurgeon. Brain CT scans obtained in the ED were subsequently reassessed by neuroradiologists.

Table 5. Criteria for the identification of a concussion, in the first 24h following an mTBI. Each indicator was dichotomically assessed. The presence of at least one of the following indicators was required.

Indicator	Comment
Loss of consciousness	Confirmed by a witness. Syncope excluded.
Post-traumatic amnesia	Evaluated by a detailed depiction of the events just before and after the mTBI.
Seizures	Objective post-concussion signs.
Vomiting	
Severe post-concussion symptoms	The severity of the following symptoms was registered on a scale from **0 to 4**: *Headache, nausea, loss of balance, hypersensitivity to light, hypersensitivity to noise, disorientation, blurred vision and dizziness.* The symptoms were classified as severe if any of them had an intensity ≥ 3, or if the sum of the intensity of all symptoms was ≥ 5 points.

2. The control group

Between April 2013 and August 2017, next-of-kin or companions of patients admitted to the Neurosurgery Department of Vall d'Hebron Research Institute—for completing clinical studies or scheduled surgery— were invited to enroll in a control group for the mTBI study. The recruitment was made in a general neurosurgical department with a wide variety of diseases that require surgery (hydrocephalus, lumbar or cervical disk surgery, brain tumors, etc.), and no participants were related to TBI patients admitted in our center. Of the candidates interested in participating, those who met inclusion criteria 1 and 2 and none of the exclusion criteria stated in the mTBI selection paragraph. In a first step, we used an active approach of selecting healthy volunteers matched by gender, age, and years of education with a known cohort of patients with mTBI. However, the 1:1 matching process was not attained in some cases (for example, for young patients with low education levels) and eventually the selection criteria were limited to the ones already stated, regardless of the composition of the mTBI group.

All patients and controls signed informed consent forms approved by Vall d'Hebron University Hospital's Ethics Committee (PR-AG-47-2013).

3. Procedures

The aim of the study was that all participants would undergo a broad set of procedures that included standardized concussion assessment, detailed neuropsychological examination and blood sampling, for biomarkers and genetic profiling. MRI scanning was performed in a subgroup of patients with mTBI and in all healthy volunteers. The members of the control group were evaluated once. Three assessment sessions were scheduled for the patients; an initial examination, in the first 24h following mTBI, the first follow-up visit, in the first 2 weeks after the mTBI, and the second follow-up visit, at 3 months after the mTBI.

For the selection of tools included in the assessment battery and of the variables in the data collection, the recommendations of the guideline for TBI assessment designed by the Common Data Elements (CDE) Task Group of the National Institute of Neurological Disorders and Stroke (NINDS) were followed (Thurmond et al., 2010; Wilde et al., 2010).

For all participants, sociodemographic data (age, sex, educational level) and relevant medical history (previous TBI and/or chronic illnesses) were recorded. History of alcohol/drug use disorder and recent consumption was noted using Alcohol, Smoking and Substance Involvement Screening Test (ASSIST v3.0,) In the group with mTBI, the following trauma-related variables were also documented: type of accident; mechanism of injury; presence and duration of LOC - when witnesses were available; duration of PTA, assessed by a detailed interview; injury sustained under the effects of psychoactive substances (detection in urine during the early ER examination); Injury Severity Score (Baker, O'Neill, Haddon, & Long, 1974).

3.1. Standardised concussion assessment

Patients were assessed with the Sport Concussion Assessment Tool 2 (SCAT2, McCrory et al., 2009) within 24 h of the mTBI during their stay at the ED's Neurotraumatology Unit and subsequently during the follow-up visits. The SCAT2 is a brief and easy-to-use tool that puts together subjective reporting and objective measuring of cognitive deficits and post-concussion signs. SCAT2 has various subsections, including a 22-item self-reported symptom checklist in which each symptom's severity is rated in a Likert scale from 0 to 6 that produces a maximum symptom severity score of 132. Additional components include a 2-item physical signs score (LOC, balance difficulties), the Glasgow Coma Scale, a modified Balance Error Scoring System (mBESS), a coordination examination and a cognitive SAC that evaluates memory, orientation and concentration. The Maddocks Score is included for sideline assessment only, not suitable for follow-up examinations, and it is not included in the total SCAT score. Although the SCAT2 was initially designed for sideline examination in sports-related concussion it has been increasingly used in the clinical setting, as it is quick to administer and addresses multiple dimensions. (Alla et al., 2009; Luoto, Silverberg, et al., 2013).

3.2. Neuropsychological assessment

Cognitive function was assessed using a neuropsychological battery testing attention, memory, information processing speed, and complex executive functions (**Table 6**). In brief, for the estimation of premorbid intelligence levels the Word Accentuation Test (*Test de acentuación de palabras*, TAP, Del Ser, González-Montalvo, Martínez-Espinosa, Delgado-Villapalos, & Bermejo, 1997) was selected. It was designed as the equivalent Spanish test for the National Adult Reading Test (NART). Although it is not as frequently used as the Vocabulary subtest of the Wechsler Adult Intelligence Scale (WAIS), it has been seen to correlate well with the WAIS score and it is considerably less time-consuming (Del Ser et al., 1997). Learning, immediate and delayed recall, and recognition were examined with the Rey's Auditory Verbal Learning Test (RAVLT, Rey, 1964) and the Brief Visual Memory Test–Revised (BVMT-R, Benedict, 1997). RAVLT also allowed for the assessment of resistance to interference. Both these tests offer multiple equivalent versions for repeated applications. The protocol included the Processing Speed Index and two of the three subtests of the Working Memory Index, from the WAIS, 3rd edition (WAIS-III, Wechsler, 2004). Block design was applied for performance visuomotor skills assessment.

Several traditional tests were aimed at establishing a potential executive dysfunction. In addition to visual attentional and cognitive processing speed, Trail Making Test [TMT, (Strauss, Sherman, Spreen, & Spreen, 2006)] assesses mental flexibility. The Controlled Oral Word Association Test [COWAT, (Strauss et al., 2006)] requires the generation of words by phonemic or semantic cue under a time constraint, and in addition to efficient lexical access it requires initiative and verbal production it relies on the ability to. The Stroop Color and Word test (Golden, 2007) measures selective attention, processing speed, cognitive flexibility and inhibition by combining timed scores for reading color names, naming colors, and naming colors written in conflicting ink (e.g. pronouncing "red" while seeing the word "green" printed in red ink). It is based on the finding that it takes longer to denominate color blocks than to read the same color names, and even longer to do the same while ignoring the written name of a distinct color–as attending to the lexical features of words is automatic while attending to the lexical associate of colors is not, although recognizing colors is even faster than reading. Conner's Continuous Performance Test v5.2 (CPT, Conners, 2004), representing the gold standard for assessing sustained attention, was comprised in the protocol. Importantly, it provides a computerized measure of processing speed, with response times in milliseconds, and additional variables that indicate disinhibition.

Effort during examination was tested using the Test of Memory Malingering (TOMM, Tombaugh, Vilar-López, García, & Puente, 2011). Patients also completed the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 2018), as anxiety and depression can influence cognitive profiles.

The remaining tests included in the protocol are presented in detail in the Supplementary Material, Section C.

Table 6. List of tests included in the neuropsychological assessment battery

Target	Test
Premorbid intelligence	Word Accentuation Test (Del Ser et al., 1997)
Memory	Rey's Auditory Verbal Learning Test (Rey, 1964) Brief Visual Memory Test–Revised (Norman et al., 2011)
Attention – speed processing	Trail Making Test (Strauss, Sherman, Spreen, & Spreen, 2006), Part A Conners' Continuous Performance Test-II, v.5.2 (Conners & Staff, 2000) Digit–symbol Coding and Symbol Search from the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III, Wechsler, 2004) Digit span (forward)–WAIS-III
Executive functioning	Controlled Oral Word Association Test (Strauss et al., 2006) Trail Making Test, Part B Letters and numbers–WAIS-III Digit span (backward)–WAIS-III Tower of London DX (Culbertson & Zillmer, 2001) Stroop test (Golden, 2007) Wisconsin Card Sorting Test, Version 4 (Heaton, 2003) Delay Discounting Test (Kirby & Marakovic, 1996) Reading the Mind in the Eyes Test (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001)
Visuospatial skills	Block design – WAIS-III
Effort during assessment	Test of Memory Malingering

In all tools, the raw scores were considered variables of interest, together with the several indexes as recommended by the literature (for example, a supplementary index for Trails Making Test is defined as the difference between the two scores divided by the score of the first part). The duration of the complete cognitive examination was approximately 120 min, including a 5 to 10-min rest period, if needed.

3.3. Neuroimaging

By hospital protocol, all patients with mTBI underwent CT scanning at the moment of their hospital arrival. In a subgroup of cases, an MRI study was performed within 14 days of the mTBI. This procedure was also performed for all healthy volunteers, within 1 year of the clinical evaluation, although generally it was done within 8 weeks (median 22 days, range 1–333 days). The MRI study was performed using a SIEMENS Magnetom Trio Tim syngo 3T MRI scanner at Hospital Clínic de Barcelona's Centre for Diagnostic Imaging. Images were analyzed by a neuroradiology expert who was not a member of the study team. For each patient, a high-resolution, T1-weighted structural image (3D magnetization-prepared rapid gradient echo [MP-RAGE]), fluid attenuation inversion recovery (FLAIR) and T2-weighted gradient echo sequences were obtained. Microbleeds were identified using susceptibility-weighted imaging (SWI). Sequences were obtained in the same order for all participants.

3.4. Biochemical and genetic profiling

One blood extraction was scheduled for all participants. For the patients, the samples were extracted during their stay in the Neurotraumatology Unit, as soon as possible after their arrival at the ED (i.e. in the first 24h after the mTBI).

The extraction of a 4 mL blood sample was performed in vacutainers with a separator gel for serum (#454058, VACUETTE® Z Serum Sep Clot Activator, Greiner Bio-One GmbH, Austria). Within 30 to 60 min of extraction, the sampling tube was centrifuged at room temperature at 4000 rpm for 10 minutes. The supernatant was stored in 300 µL aliquots at -80° C until analysis. Repeated freeze-thaw cycles were avoided.

Target proteins were analyzed with enzyme-linked immunosorbent assay (ELISA) or Multiplex commercial kits, according to the instructions provided by the supplier. See **Table 7** for more detailed information.

The samples for genetic identification were extracted in 4.5mL vacutainers coated with ethylenediaminetetraacetic acid (EDTA) (#454223, VACUETTE®, K3EDTA, Greiner Bio-One GmbH, Austria). Within 30 to 60 min of extraction, the sampling tube was stored at -80° C. The DNA extraction was performed with QIAamp DNA Blood Mini Kit Quiagen (#51104, Qiagen,

Valencia, Spain). Samples were then stored in 96 Multiply PCR-Plate neutral (#72.1979.102, Sarstedt AG& Co., Nümbrecht, Germany) and shipped to the UK facility of LGC Genomics Ltd., where the genotyping services were carried out.

4. Statistical analysis

Descriptive statistics were obtained for each variable. Mean and standard deviation were used to describe continuous variables with normal distribution and the median, maximum, and minimum values for continuous variables that were not normally distributed. The Shapiro-Wilk test and inverse probability plot were used to test whether data followed a normal distribution. Percentages and sample sizes were used to summarize categorical variables. To compare between-group differences (in categorical variables) $\chi 2$ statistics or the Fisher exact test were used as appropriate. Between-group differences were determined by an independent 2-sample t-test or the Mann–Whitney U test, depending on assumptions on statistical distribution. In order to better describe the magnitude of the differences identified, effect size was calculated with the correlation coefficient *r* or Cohen's *d*. To correlate 2 continuous variables, the most conservative Kendall tau (for data that did not follow a normal distribution) or Pearson correlation test (for data following a normal distribution) was used. Unless otherwise specified, differences were considered statistically significant when $p < 0.05$.

In most analyses, all variables were analyzed using versions 22 or 24 of the SPSS statistics package (Chicago, Illinois, USA). In some instances that were duly noted, statistical analyses were carried out with Microsoft enhanced R distribution (Microsoft R Open 3.4.3, Microsoft corporation 2017, https://mran.microsoft.com) and the integrated development environment R Studio v1.1.142 (RStudio, Inc., Boston, MA, USA; http://www.rstudio.com). The following R packages were used in the analysis: XLConnect 0.2.13, gmodels 2.16.2, dplyr 0.7.2, rcompanion 1.10.1, caret 6.0.76, and partykit.

Table 7. Technical characterization of the kits used for biomarker detection.

Biomarker	Kit Type	Dilution	Detection range	Supplier	Reference nº
S100β	ELISA	1:1	2.7 – 2000 pg/mL	EMD Millipore Corporation	#EZHS100B-33K
VEGF-A	Multiplex	1:3	13.7 - 10,000 pg/mL	EMD Millipore Corporation	#HAGP1MAG-12K
Lep	Multiplex	1:3	137.2 - 100,000 pg/mL	EMD Millipore Corporation	#HAGP1MAG-12K
vWF	Multiplex	1:40,000	0.244 - 1,000 ng/mL	EMD Millipore Corporation	#HCVD3MAG-67K
CRP	Multiplex	1:40,000	0.012 - 50 ng/mL	EMD Millipore Corporation	#HCVD3MAG-67K
IL-1b	ELISA	1:1	3.2 – 10,000 pg/mL	eBioscience Inc	#BMS224HS
IL-1b	ELISA	1:1	0.16 - 10.0 pg/mL	EMD Millipore Corporation	#HCYTOMAG-60K
Casp-1	ELISA	1:1	12.5 - 800 pg/mL	Cusabio Biotech Co	#CSB-E13025h

Abbreviations: Casp-1: caspase 1; CRP: C-reactive protein; IL-1β: interleukin 1β; Lep: leptin; VEGF-A: vascular endothelial growth factor A; vWF; von Willebrand factor.

IV. Results

This section is comprised by the following studies:

STUDY I. Rădoi A, Poca MA, Cañas V, Cevallos JM, Membrado L, Saavedra MC, Vidal M, Martínez-Ricarte F y Sahuquillo J. *Alteraciones neuropsicológicas y hallazgos neurorradiológicos en pacientes con conmoción cerebral postraumática. Resultados de un estudio piloto*. Neurología. Sep 2018; 33(7): 427—437

Original in Spanish.
For the English version, consult the Supplementary Material, Section A.

STUDY II. Rădoi A, Poca MA, Gándara D, Castro L, Cevallos M, Pacios ME y Sahuquillo J. *The Sport Concussion Assessment Tool (SCAT2) for evaluating civilian mild traumatic brain injury. A pilot normative study*. PLoS ONE. Feb 2019; 14(2): e0212541. (IF 2,776 in 2018)

Considerations by study

In the first study, 41 participants with mTBI and 28 healthy volunteers were included. The results include data regarding neuropsychological assessment, standardized concussion evaluation and findings of three MRI sequences–MPR-age, SWI and FLAIR.

In the second study, the control group was included in its entirety ($N = 60$) for investigating concussion-like symptoms in the general population with no history of head injury. Data from the application of SCAT2 and HADS were used in the analysis. Moreover, descriptive MRI findings were also included in the paper.

Section B of the Supplementary Materials includes the third study, in preparation, in which 82 patients with mTBI and 60 healthy volunteers were included in an analysis concerning protein blood biomarkers. Biochemical serum determinations were used to assess the predictive power of certain proteins for persistent post-concussion symptoms. The longitudinal profile of PPCS was assessed in relation with the normative data published in the previous study.

Furthermore, neuropsychological profile on the entire mTBI cohort and results of the extended protocol are presented in the Supplementary Material, Section C.

Genetic profiling and the risk analysis between carriers and non-carriers are presented in the Supplementary Material, Section D.

Neurología. 2018;33(7):427—437

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ORIGINAL

Alteraciones neuropsicológicas y hallazgos neurorradiológicos en pacientes con conmoción cerebral postraumática. Resultados de un estudio piloto

A. Rădoi[a], M.A. Poca[a,b,*], V. Cañas[a], J.M. Cevallos[b], L. Membrado[c], M.C. Saavedra[c], M. Vidal[a], F. Martínez-Ricarte[a,b] y J. Sahuquillo[a,b]

[a] *Unidad de Investigación de Neurotraumatología y Neurocirugía (UNINN), Institut de Recerca Vall d'Hebron (VHIR), Universitat Autònoma de Barcelona, España*
[b] *Servicio de Neurocirugía, Hospital Universitario Vall d'Hebron, Barcelona, España*
[c] *Urgencias de Neurotraumatología, Hospital Universitario Vall d'Hebron, Barcelona, España*

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PALABRAS CLAVE
Traumatismo craneoencefálico leve;
Déficits neuropsicológicos;
Lesión axonal difusa;
Sport Concussion Assessment Tool 2;
Susceptibility weighted imaging;
Síndrome posconmocional

Resumen
Introducción: Los traumatismos craneoencefálicos leves (TCE-L) han sido tradicionalmente considerados acontecimientos sin repercusiones cerebrales significativas, cuya sintomatología remite espontáneamente en unos días. Sin embargo, estos hechos son cada vez más cuestionados. Este estudio pretende objetivar la existencia de alteraciones cognitivas precoces en una serie de pacientes con TCE-L y relacionar los hallazgos con distintos marcadores de lesión cerebral.
Métodos: Estudio prospectivo de una cohorte de pacientes con un TCE-L valorados de forma consecutiva durante 12 meses. De un total de 1.144 pacientes, se seleccionó a 41 (3,7%) que habían presentado una conmoción cerebral. Además de la valoración clínica habitual y de la práctica de una tomografía computarizada (TC) cerebral, los pacientes fueron estudiados mediante un test estandarizado para síntomas posconmocionales en las primeras 24 h después del TCE-L y al cabo de 1-2 semanas y, coincidiendo con la segunda valoración, mediante una batería neuropsicológica. Los resultados se compararon con los de un grupo de 28 voluntarios sanos de características parecidas. En 20 pacientes se practicó una resonancia magnética (RM) craneal.
Resultados: En este análisis exploratorio, la memoria y el aprendizaje verbal fueron las funciones cognitivas más afectadas después del TCE-L. Siete de los 20 pacientes con TC cerebral normal presentaron alteraciones estructurales visibles por RM, que en 2 casos fueron compatibles con la presencia de lesión axonal difusa.

* Autor para correspondencia.
Correo electrónico: pocama@neurotrauma.net (M.A. Poca).

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Conclusiones: Los resultados de este estudio piloto sugieren la presencia de alteraciones cognitivas precoces y lesiones cerebrales estructurales en un porcentaje no despreciable de pacientes que han presentado una conmoción cerebral recuperada después de un TCE-L.

KEYWORDS
Mild traumatic brain injury;
Neuropsychological alterations;
Diffuse axonal injury;
Sport Concussion Assessment Tool 2;
Susceptibility-weighted imaging;
Post-concussion syndrome

Neuropsychological alterations and neuroradiological findings in patients with post-traumatic concussion: Results of a pilot study

Abstract
Introduction: Mild traumatic brain injury (mTBI) has traditionally been considered to cause no significant brain damage since symptoms spontaneously remit after a few days. However, this idea is facing increasing scrutiny. The purpose of this study is to demonstrate the presence of early cognitive alterations in a series of patients with mTBI and to link these findings to different markers of brain damage.
Methods: We conducted a prospective study of a consecutive series of patients with mTBI who were evaluated over a 12-month period. Forty-one (3.7%) of the 1144 included patients had experienced a concussion. Patients underwent a routine clinical evaluation and a brain computed tomography (CT) scan, and were also administered a standardised test for post-concussion symptoms within the first 24 hours of mTBI and also 1 to 2 weeks later. The second assessment also included a neuropsychological test battery. The results of these studies were compared to those of a control group of 28 healthy volunteers with similar characteristics. Twenty patients underwent an MRI scan.
Results: Verbal memory and learning were the cognitive functions most affected by mTBI. Seven out of the 20 patients with normal CT findings displayed structural alterations on MR images, which were compatible with diffuse axonal injury in 2 cases.
Conclusions: Results from this pilot study suggest that early cognitive alterations and structural brain lesions affect a considerable percentage of patients with post-concussion syndrome following mTBI.

Introducción

Los traumatismos craneoencefálicos (TCE) constituyen un problema de elevada prevalencia, tanto en las sociedades industrializadas como en países en vías de desarrollo, con una incidencia estimada entre 150 y 250 casos al año por cada 100.000 habitantes[1]. Un 10% de los TCE son graves (puntuación en la escala de coma de Glasgow [ECG] $\leq$ 8), un 10% moderados (puntuación en la ECG entre 9 y 13) y un 80% de los afectados presentan un TCE leve (TCE-L), con una puntuación de 14 o 15 en la ECG[2].

Tradicionalmente, se ha concedido poca importancia al estudio de las repercusiones de los TCE-L, al considerarse que se trata de un problema esencialmente reversible, sin patología cerebral detectable y con pocas o ninguna secuela residual. Sin embargo, en los últimos años los resultados de múltiples estudios cuestionan este hecho. En el contexto hospitalario, los protocolos habituales de manejo de los TCE-L establecen que cuando estos pacientes presentan una TC cerebral normal pueden ser dados de alta hospitalaria, frecuentemente sin seguimiento clínico. No obstante, existe evidencia reciente de que hasta un 25% de los TCE-L con tomografía computarizada (TC) normal presentan alteraciones en la resonancia magnética (RM) craneal[3].

Además de la puntuación en la ECG (14-15), los criterios diagnósticos que se contemplan tradicionalmente en el diagnóstico de los TCE-L son la pérdida de consciencia (PDC) —que debe ser inferior a 30 min— y la presencia de una posible amnesia postraumática (APT) de duración inferior a 24 h. Cuando alguno de estos criterios está presente, se considera que el paciente presenta una «conmoción cerebral»[4]. Las consecuencias de un TCE-L pueden ser muy variables y van desde una ausencia absoluta de síntomas residuales hasta la presencia de un cortejo sintomático florido que incluye cefaleas, mareos, náuseas, inestabilidad de la marcha, irritabilidad, alteraciones de memoria o dificultades de concentración. Tres meses después del traumatismo, aproximadamente un 30% de los afectados sigue sin recuperarse *ad integrum*[5], presentando lo que se conoce como un síndrome posconmocional (SPC)[6].

A pesar de los avances en las técnicas de identificación del daño cerebral, la mayoría de los estudios reconocen la existencia de un porcentaje de personas que después de un TCE-L presenta síntomas persistentes e incapacitantes, en ausencia de alteraciones evidentes en las pruebas de neuroimagen. Esto explica porque muchos autores consideran que la lesión cerebral puede no ser la única causa de las alteraciones a largo plazo detectadas en algunos pacientes

después de un TCE-L. En estos casos, las secuelas residuales podrían estar influidas por una serie de condicionantes como rasgos de personalidad, enfermedades mentales o sistémicas preexistentes, comorbilidad asociada (dolor crónico, trastornos ansioso-depresivos, etc.), factores sociopsicológicos y la implicación del paciente en algún tipo de reclamación o demanda judicial[7].

Según los paradigmas clásicos, la gran mayoría de las alteraciones neuropsicológicas secundarias a un TCE moderado o grave pueden explicarse por la localización de las lesiones cerebrales[8]. Sin embargo, en ausencia de lesiones focales evidentes en la TC cerebral, las disfunciones cognitivas de estos pacientes pueden deberse a la desconexión de diversas estructuras anatómicas cerebrales, debido a la presencia de una lesión axonal difusa (LAD)[5]. Este fenómeno puede constituir también el sustrato que explica la presencia de síntomas y alteraciones cognitivas residuales en algunos pacientes que han presentado un TCE-L. Entre las consecuencias neuropsicológicas más frecuentes asociadas a los TCE-L se encuentran alteraciones en la velocidad de procesamiento de la información, atención y memoria[9-12].

Para facilitar el diagnóstico de posibles lesiones estructurales en los TCE-L, los protocolos de RM más recientes incluyen secuencias de ponderación de la susceptibilidad de los tejidos (SWI, por su nomenclatura en inglés, *susceptibility weighted imaging*)[13]. El SWI es una técnica extremadamente sensible a elementos paramagnéticos, especialmente útil en la identificación de microhemorragias, habiéndose constatado en algunos estudios que esta técnica es muy superior (hasta 6 veces más)[14] a las secuencias potenciadas en T2* para la detección de microsangrados puntiformes que se asocian a la LAD.

El objetivo de este estudio piloto es valorar la presencia de alteraciones cognitivas y síntomas afectivos y conductuales precoces (antes de los 14 días después del traumatismo) en una serie de pacientes que han presentado una conmoción cerebral secundaria a un TCE-L respecto de un grupo control de voluntarios sanos, y explorar si existen relaciones entre los déficits cognitivos objetivados y los síntomas clínicos. De forma adicional, para determinar la severidad de la lesión encefálica, se pretende analizar la presencia de lesiones estructurales mediante RM en un subgrupo de pacientes incluidos en el estudio.

Pacientes y métodos

Pacientes y grupo control

Los pacientes incluidos en este estudio fueron atendidos en el servicio de Urgencias de Neurotraumatología del Hospital Universitario Vall d'Hebron (HUVH) entre abril del 2013 y abril del 2014. Para ser incluidos en este estudio, los pacientes debían cumplir todos los criterios de inclusión y ninguno de los de exclusión siguientes:

- *Criterios de inclusión*: *a)* edad comprendida entre los 18 y los 65 años; *b)* ser catalán y/o castellanoparlantes; *c)* haber presentado un TCE-L con una puntuación de 14 o 15 en la ECG dentro de las 24 h previas a la inclusión en el estudio; *d)* haber presentado una conmoción cerebral, identificada por la presencia de una pérdida transitoria de consciencia < 30 min —verificada mediante la presencia de algún testigo—, vómitos, crisis comiciales, APT inferior a 24 h o síntomas posconmocionales intensos (tabla 1); *e)* exploración neurológica normal, y *f)* TC cerebral normal.
- *Criterios de exclusión*: *1)* TCE previo que hubiera requerido atención hospitalaria; *2)* antecedentes de abuso crónico de sustancias psicoactivas; *3)* enfermedad psiquiátrica o neurológica conocida; *4)* enfermedad sistémica crónica con potenciales repercusiones cognitivas (insuficiencia renal, hepática, síndrome metabólico, etc.), y *5)* politraumatismo con un índice en la escala de severidad superior a 6.

La exploración neurológica de los pacientes y la interpretación inicial de la TC cerebral fueron llevadas a cabo por el neurocirujano de guardia, puesto que se trata de procedimientos rutinarios en la evaluación del TCE-L. Las TC cerebrales realizadas en urgencias fueron posteriormente revaluadas por los neurorradiólogos del HUVH.

El grupo control fue reclutado a partir de los acompañantes y familiares de los pacientes ingresados en el servicio de neurocirugía del HUVH. De las personas interesadas en formar parte del grupo control, se seleccionó a aquellas que cumplían los criterios de inclusión (a) y (b)

Tabla 1 Criterios empleados para la identificación de una conmoción cerebral en pacientes atendidos en las primeras 24 h después de un traumatismo craneoencefálico leve (TCE-L). En el presente estudio se exigía presencia de por lo menos uno de los siguientes indicadores, clasificados de manera dicotómica (sí/no)

Indicador	Comentario
Pérdida de consciencia	Requería la confirmación por un testigo. Debe diferenciarse de episodios sincopales
Amnesia postraumática	Evalúa la evocación detallada de los acontecimientos de justo antes y después del TCE-L
Crisis Vómitos	Signos posconmocionales objetivos
Síntomas posconmocionales severos	Registro de la severidad de los siguientes síntomas, mediante una escala de 0 a 4: Cefalea, náuseas, sensación de inestabilidad, hipersensibilidad a la luz, hipersensibilidad a los ruidos, desorientación, visión borrosa y mareos Los síntomas se clasificaron como severos si cualquiera de ellos tenía una intensidad $\geq$ 3, o la suma de la intensidad de todos los síntomas era $\geq$ 5 puntos

Tabla 2 Lista de pruebas de la batería de evaluación neuropsicológica

Objetivo de su aplicación	Nombre de la prueba
Memoria	Test de aprendizaje verbal auditorio de Rey[19]
	Brief Visual Memory Test-Revised[20]
Atención y velocidad de procesamiento	Trail MakingTest[21], Parte A
	Conner's Continuous Performance Test-II, v. 5.2[22]
	Subtest Clave de números de la batería Wechsler de inteligencia para adultos (WAIS-III)[23]
	Subtest Búsqueda de símbolos de la WAIS-III
Funciones ejecutivas	Test oral de asociación controlada de palabras[21]
	Trail Making Test, Parte B
	Subtests de memoria de trabajo de la WAIS-III (Letras y números; Dígitos)
Valoración del esfuerzo	Test of Memory Malingering[17]

y ninguno de los criterios de exclusión (1-4) antes mencionados. Además de los criterios anteriores, se seleccionaron aquellos casos con edades y nivel de estudios paralelos a las de los pacientes. El grupo control final quedó constituido por 28 voluntarios (18 varones y 10 mujeres), con una mediana de edad de 29 años (rango intercuartil [RIC] 21, mínimo: 18, máximo: 64).

Todos los pacientes y los participantes del grupo control firmaron el consentimiento informado aceptado por el Comité de Ética del HUVH (PR-AG-47-2013).

Procedimientos de evaluación y seguimiento

Además de la valoración clínica inicial, todos los pacientes fueron evaluados dentro de las primeras 24 h del traumatismo. Adicionalmente, 34 pacientes fueron estudiados en un segundo tiempo, dentro de las 2 primeras semanas post-TCE. Un subgrupo de 20 pacientes se exploró también mediante RM craneal (14 varones y 6 mujeres, con una mediana de edad de 29, RIC 21, mínimo: 18 y máximo 64). Todos los participantes del grupo control fueron valorados en una ocasión.

Evaluación estandarizada de la conmoción

Durante su estancia en Urgencias de Neurotraumatología, dentro de las primeras 24 h después del TCE-L, y en una segunda ocasión, coincidiendo con la exploración neuropsicológica, los pacientes fueron evaluados mediante la prueba *Sport Concussion Assessment Tool Second Edition* (SCAT2)[15]. El SCAT2 es una herramienta de evaluación estandarizada diseñada para medir los efectos agudos de las conmociones cerebrales producidas en un contexto deportivo. Este test registra la presencia de síntomas posconmocionales, la pérdida de consciencia (debe ser confirmada por testigos), la puntuación en la ECG y exige una evaluación del equilibrio y coordinación, así como una valoración cognitiva mediante el *Standardized Assessment of Concussion* (SAC). El SAC incluye la evaluación de la orientación temporal, memoria —inmediata y diferida— y concentración. El SCAT2 constituye una herramienta especialmente útil en el ámbito clínico debido a sus características de valoración multidimensional y a su brevedad[16]. Las puntuaciones máximas son de 30 puntos para el SAC y de 100 puntos para el SCAT2 total.

Valoración neuropsicológica

Todos los participantes fueron evaluados neuropsicológicamente en una ocasión y en el caso de los pacientes se realizó dentro de las primeras 2 semanas después del TCE-L. Para la evaluación de las funciones cognitivas se empleó una batería neuropsicológica específica formada por pruebas de valoración de la atención, memoria, velocidad del procesamiento de la información y funciones ejecutivas complejas (tabla 2). Para la elección de las pruebas se siguieron las recomendaciones del National Institute of Neurological Disorders and Stroke americano[2]. Los test seleccionados forman parte del *Core Data Elements* recomendado para el estudio de este tipo de pacientes[2]. Además, los pacientes realizaron una prueba de valoración de esfuerzo (TOMM)[17] para identificar posibles intentos de simulación de síntomas o una mera falta de colaboración durante la exploración, y respondieron a un cuestionario de sintomatología ansioso-depresiva (HAD)[18], dado que la presencia de estos síntomas puede influir en los perfiles cognitivos. La exploración cognitiva completa requería un tiempo aproximado de 120 min e incluía un periodo de 5-10 min de descanso, si era necesario. Estos estudios se llevaron a cabo por investigadoras específicamente formadas en neuropsicología (AR y VC).

Evaluación neurorradiológica mediante resonancia magnética

Los estudios por RM se realizaron en un equipo SIEMENS Magnetom TrioTim syngo 3-tesla, en el Centro para el Diagnóstico por Imagen del Hospital Clínic de Barcelona y fueron analizados por una colabora externa al proyecto, experta en neurorradiología (NB). En cada caso, se obtuvo una imagen estructural potenciada en T1, de alta resolución (3 D *Magnetization Prepared Rapid Gradient Echo*, MP-RAGE), además de secuencias mediante los protocolos *Fluid Attenuated Inversion Recovery* (FLAIR) y de gradiente *echo* en T2. Para la identificación de microsangrados se adquirieron secuencias de SWI. El orden de las secuencias fue el mismo para todos los participantes y las exploraciones se realizaron antes de 14 días desde el TCE-L.

Análisis estadístico

El análisis de todas las variables se realizó mediante el paquete estadístico SPSS (versión 22, SPSS, Chicago, Illinois, EE. UU.). Debido a que la gran mayoría de las variables no siguen una distribución normal, la comparación y el análisis

de asociaciones entre las diferentes variables registradas se realizaron mediante pruebas no paramétricas (test de la ji al cuadrado y el test de Mann-Whitney-Wilcoxon).

Con respecto a la evaluación neuropsicológica, donde el análisis implica muchas variables relacionadas, es conocido que la probabilidad de error de tipo I aumenta al realizar comparaciones estadísticas múltiples y se podría argumentar el uso de la corrección de Benjamini y Hochberg[24] para la tasa de descubrimientos falsos o directamente un p-valor más conservador de 0,01. No obstante, el tamaño muestral pequeño reduce considerablemente el poder estadístico. Por lo tanto, se decidió seguir un abordaje estadístico más liberal, asumiendo en la interpretación de todos los resultados presentados una probabilidad de error del 5%. Para describir mejor la intensidad de las diferencias estadísticamente significativas encontradas, se calculó el tamaño del efecto mediante el indicador r.

Resultados

Durante el periodo de estudio, el Servicio de Neurocirugía atendió a 1.144 pacientes con el diagnóstico de TCE-L. La mayor parte de estos pacientes tenían una edad avanzada o habían presentado un traumatismo craneal banal, sin pérdida de consciencia, ni APT ni otros síntomas relevantes asociados. De los 1.144 pacientes, se seleccionó a un total de 41 (16 mujeres y 25 varones, con una mediana de edad de 34 años, RIC de 24 y un rango de 18-64 años) (fig. 1) que cumplían los criterios de inclusión y ninguno de los de exclusión. En todos ellos se realizó una evaluación cognitiva breve y el registro de síntomas posconmocionales el mismo día del traumatismo mediante el SCAT2 y los resultados se compararon con los del grupo control. No obstante, 7 de estos 41 pacientes (17%) no acudieron a la visita de seguimiento, por lo que la valoración cognitiva extensa de seguimiento se redujo a los 34 pacientes restantes (12 mujeres y 22 varones, con una mediana de edad de 32,5 años, RIC 23, mínimo de 18 y máximo de 64).

Aplicación del *Sport Concussion Assessment Tool 2* y sintomatología ansioso-depresiva

Como era esperable, a pocas horas del TCE-L, los pacientes presentaron un número significativamente mayor de síntomas y una mayor gravedad de los mismos que los participantes sanos ($z = -4{,}44$, $p < 0{,}001$, $r = 0{,}53$ y $z = -4{,}88$, $p < 0{,}001$, $r = 0{,}58$, respectivamente; tabla 3). Asimismo, el indicador global del SCAT2 mostró diferencias significativas entre los 2 grupos ($z = 3{,}46$, $p < 0{,}001$, $r = 0{,}43$).

En la valoración clínica de seguimiento realizada varios días después del traumatismo, el número y la gravedad de los síntomas, así como el valor total del SCAT2, seguían mostrando diferencias estadísticamente significativas entre los pacientes traumáticos y los del grupo control ($z = -3{,}45$, $p < 0{,}001$; $r = 0{,}44$; $z = 3{,}22$, $p = <0{,}001$; $r = 0{,}41$; $z = 2{,}80$, $p = 0{,}005$, $r = 0{,}36$, respectivamente). En cambio, los resultados del cuestionario específico para síntomas de depresión y ansiedad HAD objetivaron que no existían diferencias significativas entre los 2 grupos en las subescalas de ansiedad ($z = -0{,}59$, $p = 0{,}55$) y depresión ($z = -0{,}68$, $p = 0{,}50$).

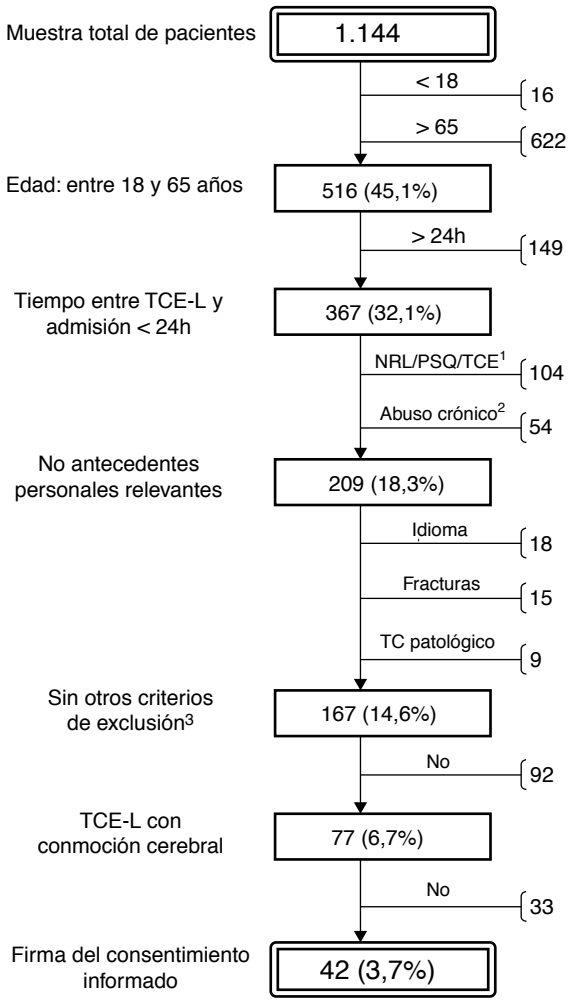

Figura 1 Algoritmo de selección de pacientes con un traumatismo craneoencefálico (TCE) leve atendidos en Urgencias de Neurotraumatología del Hospital Universitario Vall d'Hebron durante un año. Los porcentajes se refieren al número total de pacientes. Motivos de exclusión: enfermedades neurológicas, psiquiátricas o TCE previo (1); abuso crónico de alcohol o drogas (2); otros (3): conocimiento insuficiente de castellano o catalán, fracturas que requirieron ingreso y hallazgos patológicos en la TC cerebral inicial.

Valoración neuropsicológica

En los 34 pacientes que acudieron a la visita de seguimiento se realizó una valoración neuropsicológica extensa, cuyos resultados se compararon con los del grupo control (n = 28). La mayoría de los pacientes se valoraron durante la primera semana después del TCE-L (mediana: 5 días, mínimo 2 y máximo 13 días, tabla 4). La tabla 5 muestra los resultados más relevantes de la evaluación cognitiva de los 2 grupos estudiados. En la tabla puede observarse que el tamaño muestral varió entre test, dado que en los pacientes que presentaban lesiones leves de la extremidad superior dominante no se aplicaron las pruebas que requerían actividades manuales como realizar dibujos o valorar la velocidad psicomotora.

Tabla 3 Evaluación longitudinal del SCAT2 en el grupo de TCE-L y resultados en el grupo control

	TCE-L		Controles (n = 28)	Ev.1-Ev.2	Ev.1-controles	Ev.2-controles
	Ev.1[a] (n = 41) Mediana (RIC, rango)	Ev.2[b] (n = 34) Mediana (RIC, rango)	Mediana (RIC, rango)	Z (p)	Z (p)	Z (p)
N° síntomas	8 (6, 0-17)	8 (11, 0-20)	3 (4, 0-11)	0,13 (0,89)	4,44 (< 0,001)***	3,45 (< 0,001)***
Gravedad	19 (28, 0-71)	13 (36, 0-68)	5 (9, 0-31)	1,07 (0,28)	4,88 (< 0,001)***	3,22 (0,001)**
SAC	27 (4, 22-30)	25 (4, 17-29)	27 (4, 22-30)	0,04 (0,96)	2,98 (0,003)**	2,77 (0,006)**
SCAT2[c]	80 (9, 45-94)	81 (15, 55-93)	87,5 (11, 75-96)	0,66 (0,51)	3,46 (< 0001)***	2,80 (0,005)**

[a] Ev.1 representan las evaluaciones agudas, realizadas en las primeras 24 h después del traumatismo craneoencefálico leve (TCE-L).
[b] Ev.2 representan las segundas evaluaciones, realizadas a una mediana de 5 días después del TCE-L, con un mínimo de 2 y un máximo de 13 días después del traumatismo.
[c] El SCAT2 no puede calcularse en los casos en los que no se ha aplicado la evaluación del equilibrio, por lo que los valores corresponden a una n = 35 de pacientes en la exploración aguda y a una n = 31 en la exploración de seguimiento.
Ev.: evaluación; SAC: *Standardized Assessment of Concussion*; SCAT2: *Sport Concussion Assessment Tool* versión 2.
** p < 0,01.
*** p < 0,001.

La prueba que mejor permitió discriminar entre los 2 grupos fue la *Lista de aprendizaje verbal auditivo de Rey*. El rendimiento de los pacientes fue significativamente inferior que el observado en el grupo control en la capacidad de aprendizaje y la memoria verbal inmediata y diferida, con un tamaño del efecto mediano (con r > 0,3). También estaban por debajo del grupo de referencia la capacidad de aprendizaje visual y la memoria visual diferida, aunque esta última diferencia solo tendía a la significación estadística (p = 0,054). Además, los pacientes obtuvieron puntuaciones significativamente inferiores en 3 indicadores: span de memoria de trabajo, subtest de Dígitos y en el número de perseveraciones del CPT. Estas variables ofrecen información sobre aspectos de la atención y funciones ejecutivas (por el componente de memoria de trabajo y de inhibición) que aparecen alteradas a pocas semanas después del TCE-L.

Cabe destacar que debido al número elevado de variables introducidas en el análisis, cualquier estrategia estadística más conservadora, que implemente una corrección por comparaciones múltiple, establecería el umbral de significación por debajo de p = 0,003, aproximadamente, mientras que las alteraciones que se han objetivado en esta cohorte de pacientes tienen un nivel de significación de 0,005 o superior.

Resultados de la resonancia magnética

Las exploraciones mediante RM craneal se realizaron a un subgrupo de 20 pacientes, seleccionados según su disponibilidad para la realización de la prueba. La mediana de días después del traumatismo en el que se realizó la RM fue de 6, con un valor mínimo de 1 día y un máximo de 13. La tabla 6 muestra una descripción detallada de los datos demográficos de este subgrupo de 20 pacientes, junto con los hallazgos neurorradiológicos objetivados.

A pesar de que en todos los casos la TC cerebral inicial fue normal, la RM objetivó lesiones sugestivas de LAD en 2 de los 20 pacientes evaluados (10%). La figura 2 muestra las lesiones observadas en una paciente de 26 años que presentó un TCE-L con PDC y APT debido a accidente de motocicleta. De manera adicional, en otros 5 pacientes (25%) se objetivaron focos de alteraciones de la señal de etiología que podría ser traumática, aunque este hecho no pudo precisarse con certeza en todos los casos.

Discusión

Los resultados de este estudio piloto demuestran que los pacientes que han presentado una conmoción cerebral secundaria a un TCE-L pueden presentar síntomas que perduran días después del traumatismo, lo que puede interferir en la incorporación del paciente al ámbito laboral o dificultar su rendimiento académico. Más allá de los síntomas habituales de una conmoción cerebral (cefalea, vértigo...), estos pacientes pueden presentar alteraciones cognitivas objetivables mediante las herramientas adecuadas. Por último, en un porcentaje no despreciable de pacientes se identificaron alteraciones estructurales en la RM compatibles con una LAD o microsangrados, a pesar de que en todos ellos se había objetivado inicialmente una TC cerebral normal.

Tabla 4 Descriptores sociodemográficos y clínicos relevantes en los pacientes evaluados neuropsicológicamente y en el grupo control

	TCE-L (n = 34)	Controles (n = 28)	*Z* (p)
Sexo (H/M)	22/12	18/10	0,001 (0,97)[a]
Edad (años)	34 (24, 18 - 64)	29 (21, 18-64)	−0,58 (0,56)
Años de escolaridad	14 (6-22)	13,5 (8-22)	−0,32 (0,74)
Lateralidad diestro/zurdo/ambidiestro	30/3/1	26/0/2	-
Pérdida de consciencia	22 (64,70%)		
Amnesia postraumática	26 (76,47%)		
ECG (15/14)	33/1		

Mediana, rango intercuartil y valores mínimo y máximo entre paréntesis.
ECG: escala de coma de Glasgow; TCE-L: traumatismo craneoencefálico leve.
[a] Para la comparación de la distribución del sexo entre grupos se ha aplicado el test de la ji al cuadrado.

Aspectos a considerar en la inclusión de los pacientes del estudio

Uno de los hallazgos más llamativos del presente trabajo ha sido el elevado número de pacientes que acudieron al centro hospitalario para ser valorados después de presentar un TCE-L y que finalmente no fueron candidatos para el estudio. La edad fue el criterio de exclusión más frecuente. El 55% de los pacientes atendidos durante el año de desarrollo del proyecto fueron mayores de 65 años. Esta cifra concuerda con los cambios observados recientemente en los patrones epidemiológicos del TCE. A nivel mundial se ha objetivado que la edad de los pacientes traumáticos se ha incrementado de manera muy significativa y que las caídas han superado las cifras de accidentes de tráfico como causa principal del traumatismo en este grupo de edad[1]. Los estrictos criterios de cribado aplicados, elegidos específicamente para eliminar factores de confusión conocidos, redujeron el número potencial de participantes hasta el 6,9%. Por otra parte, solo el 60% de los que cumplían los criterios de inclusión accedieron a participar de forma no remunerada en el estudio. De esta forma, el porcentaje de participación final se redujo a menos del 4% de todos los pacientes atendidos durante un año en un hospital de nivel tres. En un estudio publicado en 2013, Luoto et al. advertían sobre este hecho y afirmaban que muchos de los estudios hospitalarios que se dirigen a

Tabla 5 Análisis comparativo de la evaluación neuropsicológica realizada en los pacientes evaluados y el grupo control

	TCE-L		Controles (n = 28)		
	n	Mediana (RIC, rango)	Mediana (RIC, rango)	*Z* (p)	r
TMT A	*32*	32,5 (19, 19-86)	30 (11, 13-54)	−1,632 (0,103)	0,21
TMT B	*29*	65 (47, 34-143)	61 (20, 24-150)	−0,559 (0,576)	0,07
Índice ejecutivo TMT[a]	*29*	1 (0,82, 0,36-2,59)	1,03 (0,93, 0,32-3,5)	−0,439 (0,661)	0,06
Aprendizaje verbal	*34*	49,5 (10, 27-68)	57 (12, 39-71)	−2,812 (0,005)**	0,36
M. verbal inmediata	*34*	11,5 (5, 6-15)	13,5 (3, 6-15)	−2,596 (0,009)**	0,33
M. verbal demorada	*34*	11 (4, 5-15)	13,5 (4, 8-15)	−2,802 (0,005)**	0,36
Clave de números	*31*	73 (25, 29-113)	83 (29, 48-120)	−1,693 (0,090)	0,22
Búsqueda de símbolos	*32*	38,5 (11, 15-54)	39,5 (14, 25-51)	−0,519 (0,604)	0,07
Span atencional	*34*	6 (1, 4-9)	6 (2, 4-9)	−1,282 (0,200)	0,16
Span m. de trabajo	*34*	4 (2, 2-7)	5 (2, 3-8)	−2,366 (0,018)*	0,30
Dígitos	*34*	14 (6, 7-23)	16 (4, 9-26)	−2,202 (0,028)*	0,27
Letras y números	*31*	11 (4, 5-17)	11,5 (3, 7-16)	−0,972 (0,331)	0,12
M. visual inmediata	*32*	6 (3, 1-11)	7 (6, 0-11)	−1,837 (0,066)	0,24
Aprendizaje visual	*32*	25 (9, 7-33)	29,5 (9, 7-35)	−2,483 (0,013)*	0,32
M. visual demorada	*32*	10 (4, 3-12)	11,5 (2, 4-12)	−1,925 (0,054)	0,25
Fluencia semántica	*34*	24 (8, 16-40)	24 (9, 13-37)	−0,312 (0,755)	0,04
Fluencia fonética	*34*	43 (14, 23-59)	46 (21, 20-80)	−1,196 (0,232)	0,15
Omisiones-CPT	*34*	1 (3, 0-30)	1 (3, 0-13)	−0,007 (0,994)	< 0,01
Comisiones-CPT	*34*	13 (12, 2-31)	11 (10, 1-26)	−1,711 (0,087)	0,22
Tiempo de reacción-CPT	*34*	402 (87, 306-583)	391 (48, 332-588)	−0,087 (0,931)	0,01
Perseveraciones-CPT	*34*	0 (2, 0-23)	0 (0, 0-4)	−2,292 (0,022)*	0,29

CPT: *Continuous Performance Test*; M.: memoria; TCE-L: traumatismo craneoencefálico leve; TMT: *Trail Making Test*.
[a] Índice ejecutivo TMT = (TMT B − TMT A)/TMT A.
* p < 0,05.
** p < 0,01; r = tamaño del efecto (0,5 grande, 0,3 mediano y 0,1 pequeño).

Tabla 6 Características demográficas, clínicas y neurorradiológicas de los 20 pacientes evaluados mediante RM, ordenados por la puntuación del SAC

N°	Edad/sexo	Causa TCE	Evaluación inicial (<24 h)			Hallazgos en la exploración RM
			N.° síntomas	SAC	SCAT2	
1	64/M	Caída casual	15	20	67	Afectación de SB subcortical (Fazekas 2) Pequeño quiste de cisura coroidea
2	26/M	Accidente tráfico (motorista)	10	20	49	Pequeñas lesiones en SB subcortical izquierda, algunas con microsangrado, indicativas de LAD
3	43/H	Caída de 3 m	8	20	82	Pequeñas lesiones aisladas en SB de escaso valor patológico, sin microsangrado
4	52/H	Accidente laboral	15	20	62	Alguna pequeña lesión inespecífica en SB subcortical frontal
5	20/H	Accidente deportivo	10	22	79	Sin hallazgos notables
6	50/H	Accidente de tráfico (ciclista)	2	23	86	Lesiones sugestivas de LAD de predominio frontal bilateral con microsangrados asociados
7	42/M	Accidente de tráfico (motorista)	0	25	74	Pequeño microsangrado en hemiprotuberancia izquierda. Alteración de señal en SB periventricular (Fazekas 1)
8	30/M	Caída casual	12	25	80	Sin hallazgos notables
9	28/H	Agresión	13	25	—[a]	"
10	56/M	Caída de 2 m	2	25	82	"
11	38/H	Agresión	9	26	77	Único foco de microsangrado temporal posterior
12	27/H	Accidente de tráfico (atropello)	8	26	82	Sin hallazgos notables
13	22/H	Agresión	1	26	74	"
14	27/M	Accidente de tráfico (motorista)	8	27	88	"
15	24/H	Accidente deportivo	7	27	84	"
16	46/H	Accidente de tráfico (motorista)	6	27	86	Pequeñas lesiones inespecíficas en SB subcortical y periventricular, sin focos de microsangrado
17	18/H	Accidente deportivo	9	27	84	Sin hallazgos notables
18	19/H	Accidente deportivo	7	28	83	"
19	30/H	Accidente deportivo	7	29	—	"
20	24/H	Accidente deportivo	4	30	94	"

LAD: lesión axonal difusa; RM: resonancia magnética; SAC: *Standardized Assessment of Concussion*; SB: sustancia blanca; SCAT2: *Sport Concussion Assessment Tool* versión 2.

[a] En pacientes encamados no se ha aplicado la prueba de equilibrio y no se pudo calcular la puntuación global del SCAT2.

temas específicos de investigación sobre los TCE-L reclutan muestras que pueden estar sesgadas y que sus resultados no pueden generalizarse a toda la población de traumáticos[25].

Criterios diagnósticos del traumatismo craneoencefálico leve

De acuerdo con los criterios clásicos de la Organización Mundial de la Salud en 2004[4], el diagnóstico de un TCE-L exige que el paciente presente una puntuación de 13, 14 o 15 en la ECG. Sin embargo, siguiendo criterios diagnósticos más actuales, en el presente estudio se han descartado aquellos pacientes que presentaban una puntuación de 13. Diversos autores señalan que la evolución de los pacientes con una puntuación de 13 en la ECG es más comparable con la de los TCE moderados que con la de los leves, sobre la base de indicadores de mortalidad y complicaciones[26]. Stein y Ross compararon los hallazgos en la TC cerebral inicial de un grupo de 106 pacientes que presentaban una puntuación de 13 en la ECG con la de 341 pacientes etiquetados de TCE moderado de acuerdo con los criterios clásicos (puntuaciones entre 9 y 12 en la ECG)[27]. El porcentaje de lesiones objetivadas en la TC cerebral de ambos grupos fue superponible (44,3% vs. 40,3%, respectivamente). Estos autores encontraron, además, que un 20% de los pacientes con una TC cerebral patológica requirieron intervención quirúrgica, por lo que proponen, ya en 1992, que los pacientes

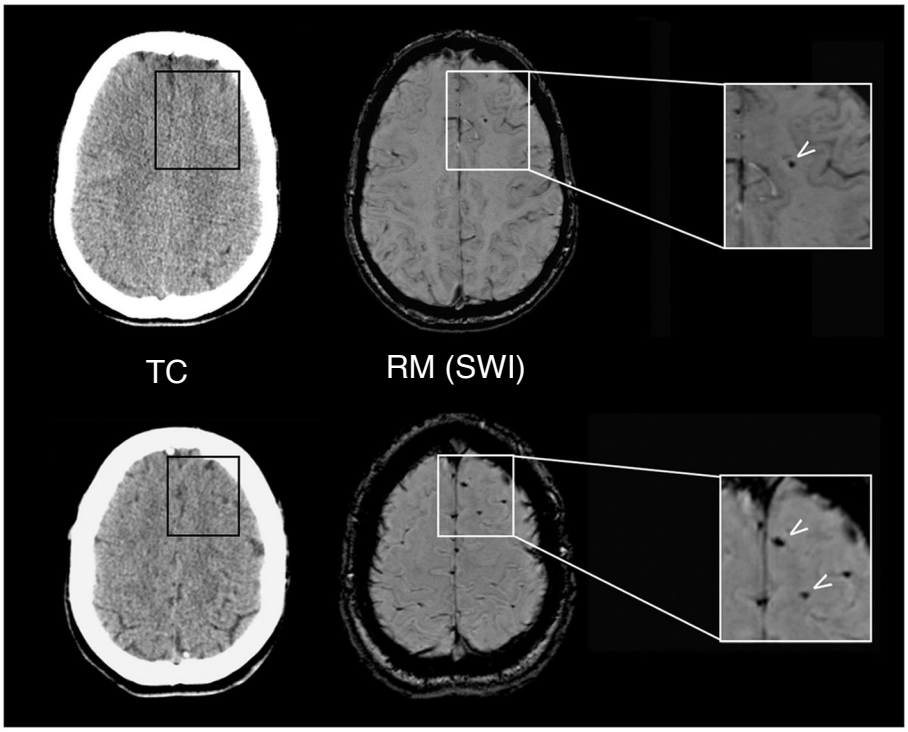

Figura 2 Hallazgos neurorradiológicos en un paciente de 26 años que presentó un traumatismo craneoencefálico leve. La tomografía computarizada (TC) cerebral, practicada a las 2 h del traumatismo, fue normal (izquierda), mientras que la resonancia magnética (RM) craneal (derecha) practicada a los 10 días objetivó focos de alteración de la señal en la secuencia *Susceptibility Weighted Imaging* (SWI) (flechas), correspondientes a microsangrados indicativos de lesión axonal difusa leve.

traumáticos con una puntuación de 13 en la ECG deberían ser reclasificados como moderados y no leves[27].

En el presente estudio también se exigía que la TC cerebral fuera normal. La exclusión de los pacientes con una exploración tomográfica patológica asegura una mayor homogeneidad de la muestra, puesto que en los pacientes con lesiones en la TC cerebral se utilizan protocolos de seguimiento distintos, más cercanos a los de los TCE moderados. La ausencia de lesiones cerebrales en los estudios convencionales que se practican en Urgencias permite centrar el estudio en el extremo más benigno de esta patología (pacientes con una puntuación de 14 o 15 y TC cerebral normal).

Valoración clínica en el traumatismo craneoencefálico leve. Herramientas actuales

Para profundizar en el estudio de por qué algunos pacientes presentan un SPC, es necesario conocer mejor la traducción clínica de los TCE-L en la fase aguda. El valor pronóstico de descriptores tradicionales, como la presencia y la duración de la PDC y de la APT, no está bien establecido y el conocimiento fisiopatológico de ambos fenómenos en el contexto de los TCE-L es aún limitado. En estos pacientes, la validez y el poder predictivo de la APT están ampliamente cuestionados, a pesar de ser un signo definitorio de la conmoción y un elemento crítico de la evaluación hospitalaria de rutina[16].

Con el objeto de registrar de forma exhaustiva la información clínica de los pacientes con un TCE-L estudiados, se utilizó una escala inicialmente diseñada para valorar traumatismos en el ámbito deportivo (SCAT2), observándose que los pacientes valorados seguían presentando un número elevado de síntomas posconmocionales al cabo de 1-2 semanas después del traumatismo, aunque su severidad tendía a reducirse respecto a la valoración inicial. Los resultados obtenidos de la aplicación del SCAT2, tanto el día del traumatismo como 1-2 semanas después, avalan el uso de esta herramienta en la monitorización estandarizada de la sintomatología posconmocional, dado que también ofrece una cuantificación global del estado cognitivo de estos pacientes. No obstante, la evaluación de los síntomas clínicos —PDC y pérdida de equilibrio— en el contexto de los TCE-L valorados es este estudio es menos fiable que en el contexto deportivo, ya que en muchos casos se basa en información proporcionada por el propio paciente o relatada por testigos. Además, la exploración del equilibrio no se puede realizar en aquellos pacientes con lesiones en extremidades inferiores que dificulten la bipedestación. Por otra parte, los resultados en la valoración del equilibrio son más variables en la población adulta general que en los jóvenes deportistas: el 10% de una muestra de población canadiense sana y aproximadamente el 65% de un grupo control finlandés obtuvieron puntuaciones bajas en las pruebas de equilibrio[16]. Estos resultados indican que el valor de este subtest de equilibrio dentro del SCAT2 debería ser reconsiderado en futuros estudios

dirigidos a validar el uso de esta escala fuera del contexto deportivo.

Resultados de la valoración neuropsicológica

A pesar de que el TCE puede alterar casi cualquier aspecto del funcionamiento encefálico, posiblemente la afectación clave de estos pacientes sea la disfunción de los sistemas frontales, donde se centran especialmente las funciones ejecutivas (capacidad de planificación y organización, memoria de trabajo, flexibilidad e inhibición de conductas, entre otras). Esta perspectiva ha hecho que autores como Chen y d'Esposito (2010)[28] y Stuss (2011)[29] definan el TCE como un trastorno del control cognitivo.

Los resultados de la evaluación neuropsicológica detallada realizada en este estudio indican que durante las primeras semanas posteriores a un TCE-L la capacidad de aprendizaje y la memoria se encuentren levemente alteradas, en comparación con los hallazgos de un grupo de control. Otros indicadores de atención, que incluyen también un componente ejecutivo puesto que requieren memoria de trabajo e inhibición, se añaden a los resultados que describen la presencia de déficits cognitivos sutiles en este grupo de pacientes.

Estos resultados son fruto de un análisis exploratorio y las diferencias entre los pacientes y el grupo de control han sido consideradas significativas estadísticamente a un $p < 0,05$, sin una corrección *post hoc* estricta. No obstante, concuerdan con los resultados de una revisión sistemática reciente, que concluyó que la asociación entre el TCE-L y los déficits cognitivos en las primeras 2 semanas es un hallazgo consistente[11]. En este periodo de seguimiento, la PDC se asoció, aunque de forma limitada, a una reducción en la velocidad de procesamiento de la información. Sin embargo, aunque casi el 70% de los pacientes incluidos en nuestro estudio habían presentado una PDC, los resultados obtenidos no confirman de manera clara la existencia de un déficit específico de velocidad de procesamiento.

Tomografía computarizada normal y lesiones en la resonancia magnética

Los pacientes con un TCE-L, y especialmente aquellos con sintomatología persistente, podrían presentar lesiones cerebrales que pueden pasar desapercibidas en la TC cerebral. La secuencia SWI identificó la presencia de lesiones traumáticas estructurales en un 10% de los 20 pacientes estudiados por RM. De forma adicional, en otros 5 casos (25% de los pacientes estudiados) se objetivaron lesiones en la RM que no se visualizaron en la TC cerebral convencional, aunque en algunos casos la etiología de estas lesiones fue incierta.

A pesar de que en otros estudios el volumen total de las lesiones que se identifican en las secuencias SWI se correlaciona con indicadores clínicos de severidad[30], no se ha establecido una relación clara entre la presencia de estas lesiones y la recuperación cognitiva después del traumatismo. Diversos estudios han objetivado que la existencia de lesiones en las exploraciones de neuroimagen de pacientes con un TCE-L se asocia a peores resultados en funciones cognitivas como la memoria. Sin embargo, debido a que la gran mayoría de las pruebas neuropsicológicas aplicadas no discriminan entre los grupos de pacientes con y sin lesiones neurorradiológicas, hay autores que opinan que estos pacientes no deberían tratarse de forma distinta[31].

La identificación de estas lesiones cerebrales regionales, que aparecen en el momento agudo del TCE-L y persisten de forma indefinida, aporta información no solo de la severidad de la lesión, sino también sobre qué sistemas neuroconductuales pueden haber sido afectados. Las exploraciones de RM avanzada mejoran la compresión de la distribución de la lesión encefálica y, en última instancia, permiten el desarrollo de estrategias de evaluación y tratamiento más efectivas, de forma análoga a como ocurre el proceso de rehabilitación de los pacientes que han presentado un accidente vascular isquémico.

En conclusión, a pesar de que este estudio presenta una serie de limitaciones, los resultados obtenidos confirman que, en contra de la creencia ampliamente extendida en el ámbito clínico, determinados TCE-L no deberían considerarse acontecimientos banales. A pesar de presentar una TC normal, los estudios de RM avanzada objetivaron que entre el 10 y el 35% de los pacientes estudiados presentaban lesiones que podían ser indicativas de una LAD. Tanto en la fase aguda como a 1-2 semanas después del traumatismo, los pacientes evaluados presentaban alteraciones del estado neurocognitivo global, en comparación con el grupo control. Los resultados de la valoración neuropsicológica indican que a medio plazo el estado cognitivo de estos pacientes sigue alterado y que presentan problemas de memoria y de atención ejecutiva. Una de las principales limitaciones del estudio es el periodo relativamente corto de seguimiento. Después de un TCE-L los síntomas podrían persistir durante meses después del traumatismo o incluso, en algunos aspectos, hacerse permanentes. Esto implica la necesidad de extender el periodo de seguimiento de los enfermos. Este aspecto deberá ser incluido en futuros estudios.

El conjunto de esta información pone de manifiesto que el manejo que suele hacerse en un medio hospitalario del paciente con un TCE-L (habitualmente valoración y alta hospitalaria sin seguimiento) puede no ser adecuado en algunos casos. El registro estructurado de la sintomatología posconmocional y la evaluación neuropsicológica aportan información muy relevante sobre las alteraciones que estos pacientes pueden presentar durante al menos las 2 primeras semanas después del traumatismo. A pesar de que es necesario ampliar la muestra de pacientes, los resultados obtenidos hasta el momento permiten afirmar que el cuestionario SCAT2 utilizado en este estudio constituye una herramienta útil, por lo que debería considerarse su incorporación en la asistencia clínica rutinaria.

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Conflicto de intereses

Ninguno de los autores presenta conflictos de intereses.

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PLOS ONE

RESEARCH ARTICLE

The Sport Concussion Assessment Tool (SCAT2) for evaluating civilian mild traumatic brain injury. A pilot normative study

Andreea Rădoi[1,2]*, Maria A. Poca[1,2,3], Darío Gándara[3], Lidia Castro[1,2], Mauricio Cevallos[3], Maria E. Pacios[4], Juan Sahuquillo[1,2,3]

1 Neurotraumatology and Neurosurgery Research Unit (UNINN), Vall d'Hebron Research Institute (VHIR), Barcelona, Spain, **2** Universitat Autònoma de Barcelona, Barcelona, Spain, **3** Department of Neurosurgery, Vall d'Hebron University Hospital, Barcelona, Spain, **4** Neurotraumatology Emergency Unit, Vall d´Hebron University Hospital, Barcelona, Spain

* aradoi@neurotrauma.net

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Abstract

Self-report measures, particularly symptom inventories, are critical tools for identifying patients with persistent post-concussion symptoms and their follow-up. Unlike in military or sports-related assessment, in general civilian settings pre-injury levels of concussion-like symptoms are lacking. Normative data are available in adolescent and college populations, but no reference data exist to guide clinical adult explorations. The purpose of this study was to use the second edition of the Sport Concussion Assessment Tool (SCAT2) to profile a cohort of 60 healthy community volunteers who had not sustained a head injury. Participating volunteers underwent MRI scanning and were evaluated with the Hospital Anxiety and Depression Scale (HADS). Participants reported a median of 3 concussion-like symptoms and the 97.5 percentile score was found at 10.5 symptoms, out of a total of 22. The median severity score was 4.9 points, and 28.9 was the upper limit of the reference interval. Only 10 participants (16.7%) did not endorse any symptom. The most frequently endorsed symptom was feeling difficulty in concentrating, with 41.7% of the sample reporting it. Age, sex and general distress, anxiety and depressive symptoms were not associated with concussion-like symptoms. Our data yielded elevated cut-offs scores for both the number of symptoms and the symptom severity. In conclusion, postconcussive-like symptoms are frequent in the general non-concussed adult population and it should be taken into account in any future models developed for screening patients at risk of developing physical, cognitive, and psychological complaints following mild traumatic injury.

Introduction

Mild traumatic injury (mTBI) and specifically concussion as a result of traffic accidents, assaults, sports, work injuries or deployed military have been acknowledged as a topic of intense public concern in the last decade.[1, 2] Concussion may translate into somatic symptoms—dizziness, nausea, headaches, etc.—and may also affect cognitive and emotional

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Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BMI, body mass index; CT, computed tomography; DTI, diffusion tensor imaging; ED, emergency department; EI, Evans index; FLAIR, fluid-attenuation inversion recovery; GCS, Glasgow Coma Scale; HADS, Hospital Anxiety and Depression Scale; LOC, loss of consciousness; M-BESS, modified Balance Error Scoring System; MP-RAGE, magnetization-prepared rapid gradient-echo; MRI, magnetic resonance imaging; mTBI, mild traumatic brain injury; PPCS, persistent post-concussion symptoms; PTA, posttraumatic amnesia; SAC, Standardized Assessment of Concussion; SCAT2, Sport Concussion Assessment Tool 2nd Edition; TBI, traumatic brain injury; WHO, World Health Organization.

functioning, and in some patients these consequences can be long-lasting.[3] Recent multidisciplinary clinical and research efforts have been addressed at improving mTBI diagnosis, tracking recovery and identifying patients at risk of experiencing persistent post-concussion symptoms (PPCS) such as headaches, dizziness, fatigue, sleep disturbances and/or cognitive problems.

The American Congress of Rehabilitation Medicine defined mTBI in 1993 as any 'traumatically-induced physiological disruption of brain function manifested at least by loss of consciousness of less than 30 minutes, posttraumatic amnesia (PTA) not greater than 24 h, any alteration of the mental status or transient or non-transient focal neurological deficits'.[4] However, the controversy regarding the operational definition of mTBI diagnosis is ongoing.[5] Some consider the term 'concussion' equivalent to mTBI and the term includes mechanically-induced brain dysfunctions at the mild end of the severity spectrum. The discussion around the implications of the use of terms 'concussion' and 'mTBI' is beyond the scope of this paper, but the reader is referred to the comprehensive review by Sharp and Jenkins.[6] For the sake of simplicity, in this paper concussion refers to any mTBI without any evidence of structural brain damage in the computed tomography (CT) scan. However, there is increasing evidence that up to 20–30% of mTBI patients with normal CT scan show significant brain changes either in structural or functional magnetic resonance imaging (MRI).[7]

Several tools and scales have been designed and used for the clinical assessment of mTBI.[8] All of them include an inventory of self-reported symptoms, frequently applied together with neuropsychological and postural equilibrium tests.[8] Although symptom reporting has become the most used strategy to predict concussion outcome, the symptoms triggered by concussion are notoriously heterogeneous and non-specific. Patients with no TBI history but presenting other conditions (chronic pain, depression, etc.) endorse many concussion-like symptoms and, in some cases, with similar severity.[9, 10] In addition, cohorts described as 'healthy' populations also endorse concussion-like symptoms at what could be considered a clinically relevant rate.[11, 12]

One of the most widely used tools in mTBI is the Sport Concussion Assessment Tool (SCAT), a scale proposed by the international Concussion in Sport group as part of a comprehensive concussion screening instrument in sports.[13, 14] The original scale contained 18 symptoms as a measure of an individual's status following TBI, in addition to other 7 items specifically designed for follow-up visits.[13] In 2009, the International Symposia on Concussion in Sport consensus statement proposed the second version of this tool (SCAT2).[15] The SCAT2 is a brief and easy-to-use tool that puts together self-reported symptoms and objective evaluation of cognitive deficits and post-concussion signs. SCAT2 has various subsections and among them a 22-item self-reported symptom checklist in which each symptom's severity is rated in a Likert scale from 0 to 6.[15] Additional components include a 2-item physical signs score, the Glasgow Coma Scale, a modified Balance Error Scoring System (M-BESS), a coordination examination and a cognitive Standardized Assessment of Concussion (SAC) that evaluates memory, orientation and concentration. Although several updated editions of the SCAT have been put into use, the symptom checklist has remained unchanged up to the most recent edition, which is SCAT5.[16]

Since 2004, the World Health Organization (WHO) Collaborating Centre Task Force on mTBI has recommended post-concussive symptoms to be assessed in conjunction with psychosocial or injury-related factors (pain, depression, anxiety, posttraumatic stress, litigation status, etc.).[17] In particular, psychological distress has been consistently linked with more severe early symptomatology and with a slower recovery.[17] As such, one hypothesis is that baseline psychological wellbeing can modulate SCAT performance. Putukian et al. have shown that, in college students, depression and anxiety levels were associated with SCAT2 baseline

higher severity scores and higher number of symptoms, although not with SAC or balance scores. In this study, athletes endorsing baseline depressive symptoms and/or anxiety reported worse symptom severity and more symptoms in the SCAT2.[18]

Although the SCAT2 was initially designed for sideline examination in sports-related concussion, it has been increasingly used in the clinical setting.[19, 20] The general approach in using the different SCAT versions is to measure the athlete's post-trauma performance in comparison with a baseline evaluation collected before season.[21] However, in civilian assessment of concussion, a baseline SCAT score is never available and therefore the same approach is useless. The aim of this study was to explore the frequency of concussion-like symptoms and their severity in a healthy civilian population. Our goal was to establish population-based symptoms and severity score thresholds that can be used in future multivariate analysis and supervised-machine learning models of non-sport mTBI studies and, furthermore, to evaluate whether the concussion-like symptoms endorsed by the cohort were influenced by age, sex or anxiety and depressive symptoms. Supervised machine-learning models that screen for patients at risk of developing persistent post-concussion symptoms could increase their accuracy—sensitivity, specificity and predictive value—by identifying the patients with concussion-like symptoms that are common in the healthy population.

Participants and methods

Setting and participants

The Traumatology Hospital at the Vall d'Hebron University Hospital is an academic tertiary referral center with a translational research program in TBI and a comprehensive neurorehabilitation facility with expertise in TBI. One of the ongoing research projects is a prospective study on the outcome of mTBI in an adult civilian cohort, in which a supervised-machine learning approach will be used to discriminate patients at high risk of developing persistent post-concussion symptoms. To avoid selecting an arbitrary cut-off outcome based on SCAT2, we carried out a pilot study to understand the baseline characteristics of a civil population and to explore the potential number of symptoms and their severity that could define a clinically relevant threshold.

Between April 2013 and August 2017, next-of-kin or companions of patients admitted to the Neurosurgery Department of our institution—for completing clinical studies or surgery—were invited to enroll in a control group for the mTBI study. The recruitment was made in a general neurosurgical department with a wide variety of diseases that require surgery (hydrocephalus, lumbar or cervical disk surgery, brain tumors, etc.), and no participants were related to TBI patients admitted in our center. Inclusion criteria were to be between 18 and 65 years of age and to proficiently speak Spanish and/or Catalan. Exclusion criteria were: 1) history of TBI, regardless of severity; 2) history of chronic abuse of psychoactive substances or alcohol; 3) known psychiatric or neurologic disorder; 4) chronic systemic disease with known repercussions on the cognitive status by itself or its treatment (cancer, kidney or liver failure, metabolic syndrome, etc.). Participants reported their education status as the highest level completed as well as the number of full-time years of study completed. The following equivalence can be established: 8 years for primary education, 10 years for secondary education, 12 or 13 years for high-school or professional studies, and 1 more year for each full-time undergraduate and postgraduate school-year. In a WHO national survey conducted in Spain in 2011–2012 among individuals aged 18 years or over, the prevalence of obesity for men and women in Spain was 18.0% and 16.0%, respectively.[22] Because of this we decided to include BMI as a baseline variable of our cohort. The body mass index (BMI), calculated as the body mass divided by the square of the body height, was used to classify the cohort into underweight (<18.5 kg/m^2),

normal-weight (between 18.5 and 24.9 kg/m^2), overweight (between 25 and 29.9 kg/m^2) or obese (>30 kg/m^2) categories. In a first step, we used an active approach of selecting healthy volunteers matched by gender, age, and years of education with a known cohort of patients with mTBI. However, the 1:1 matching process was not attained in some cases (for example, for young patients with low education levels) and eventually the selection criteria were limited to the ones already stated, regardless of the composition of the mTBI group. This study was reviewed and approved by the Ethics Committee of the Vall d´Hebron Research Institute (PR-AG-47-2013) and all participants signed a written informed consent.

Assessment procedures

The present study focuses on the concussion standardized evaluation, carried out with the **SCAT2**.[15] The structure of SCAT2 is described in **Table 1**. In brief, in the symptom checklist the respondent evaluates the presence/absence of 22 post-concussion predefined symptoms and rates each symptom intensity on a Likert scale from 0 to 6. This results in two scores: the number of endorsed symptoms at evaluation and their severity. Items evaluating orientation, working memory and verbal memory are summed up in a cognitive index known as Standardized Assessment of Concussion (SAC) score, with a minimum value of 0 and a maximum of 30 points. For the purposes of this study we used as endpoints both the total number of endorsed symptoms (0–22) and their total severity score (0–132). The symptom profile was divided into four clusters: somatic, cognitive, emotional and fatigue-sleep domains (**Table 1**). The probability of endorsing a symptom cluster is linked to the number of items it compasses (i.e. 9 for somatic versus 3 for sleep-fatigue). As the participants had not sustained any head

Table 1. The components of the Sport Concussion Assessment Test 2nd edition (SCAT2). The following editions (SCAT3 and SCAT5) preserve this structure with minor scoring modifications and new supplementary material.

Self-report symptom check-list			
Somatic	**Cognitive**	**Emotional**	**Fatigue/sleep**
• Headache	• Feeling slowed down	• More emotional	• Trouble falling asleep
• 'Pressure in head'	• Feeling like 'in a fog'	• Irritability	• Drowsiness
• Neck pain	• 'Don't feel right'	• Sadness	• Fatigue / low energy
• Nausea or vomiting	• Difficulty concentrating	• Nervous or anxious	
• Dizziness	• Difficulty remembering		
• Blurred vision			
• Balance problems			
• Sensitivity to light			
• Sensitivity to noise			
Cognitive examination *(Standardized Assessment of Concussion)*			
• *Immediate memory*: 3 trials of a list of 5 unrelated words			
• *Delayed memory*: free recall of the previously taught list			
• *Orientation*: 5 questions of time orientation			
• *Concentration*: backwards repetition of digit series and months of the year in reverse order			
Other components			
Balance examination: Modified Balance Error Scoring System (M-BESS)			
Coordination: finger-to-nose test			
Physical signs: loss of consciousness, balance difficulties			
Glasgow Coma Scale: standard neurologic evaluation			
Maddocks Score: optional—only suitable for sideline evaluation in sports-related concussion			

SCAT3: third version of Sport Concussion Assessment Test; SCAT5: fifth version of Sport Concussion Assessment Test

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trauma, the items regarding the postconcussive physical signs (loss of consciousness, balance difficulties) and the Glasgow Coma Scale were not applied and the maximum score for these components was granted in computing the SCAT2 total.

The **Hospital Anxiety and Depression Scale** (HADS) is a short self-report multiple-choice questionnaire, frequently used for screening clinically significant anxiety and depression in patients attending any general clinical setting.[23] The respondent is asked to fill in the answers in order to reflect how they have been feeling during the previous week. The anxiety and depression subscales are assessed separately. Each subscale has 7 items that can be graded from 0 to 3 points, therefore the scores range from 0 to a maximum of 21. The scores between 0 and 7 are classified as normal, between 8 and 10 as borderline, and 11 points or more as abnormal. The total HADS score ranges from 0 to 42, with higher values indicating more emotional distress. HADS's screening properties are as good as other more comprehensive instruments used for identification of anxiety and depressive disorders.[24]

In addition, participants underwent a broader set of procedures that included detailed neuropsychological assessment and blood sampling for biomarkers and genetic determination (data not presented here). The study protocol also included performing a magnetic resonance imaging (MRI) within 1 year of the clinical evaluation, although generally it was done within 8 weeks (median 22 days, range 1–333 days). The MRI scanning was performed with a SIEMENS Magnetom TrioTim syngo 3-tesla equipment; data from a high-resolution 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) protocol in addition to Fluid Attenuated Inversion Recovery (FLAIR) and echo gradient T2 sequences were assessed and informed by an expert neuroradiologist in all cases.

Statistical analysis

Descriptive statistics were obtained for each variable. The Shapiro-Wilk test and inverse probability plot were used to test whether data followed a normal distribution. The mean and the standard deviation were used to describe continuous variables that followed a normal distribution and the median, maximum, and minimum values for continuous variables that were not normally distributed. Percentages and sample sizes were used to summarize categorical variables. To compare between-group differences (in categorical variables) $\chi 2$ statistics or the Fisher exact test were used as appropriate. Between-group differences were determined by an independent 2-sample *t*-test or the Mann–Whitney U test, depending on the statistical distribution. To correlate 2 continuous variables, the Kendall tau (when data did not follow a normal distribution) or Pearson correlation test (for data following a normal distribution) were used. The threshold for statistical significance was lowered from the routine p value of 0.05 and statistically significance was considered when $p < 0.005$.[25] This decision was taken following recent suggestions by many authors to change the default p -value threshold for statistical significance from 0.05 to 0.005, in particular in pilot studies, like ours, and with small sample sizes, as the risk of reporting false positive results is higher[25, 26].

To calculate the reference intervals (RIs) for the number of symptoms endorsed and the symptom severity score, the first step was to apply the Horn's algorithm[27] to detect outliers. If Horn's algorithm detected a significant outlier, the data was considered doubtful and eliminated of the RIs calculation. To calculate the upper and lower RI limits we used the distribution-free nonparametric method described in the NCCLS and Clinical and Laboratory Standards Institute (CLSI) guidelines C28-A3 for estimating percentiles intervals[28, 29] by using the package 'referenceIntervals' for R.[30]

Statistical analyses were carried out with Microsoft-enhanced R distribution (Microsoft R Open 3.4.4, Microsoft corporation 2017, https://mran.microsoft.com) and the integrated

development environment R Studio v1.1.453 (RStudio, Inc., Boston, MA, USA; http://www.rstudio.com). The following R packages were used in the analysis: XLConnect 0.2.13, Hmisc 4.0.3, referenceIntervals 1.1.1 and car 2.1.6.

Results

Participants

The group of healthy volunteers consisted of 60 participants, out of which 38 (63.3%) were men, whose socio-demographic characteristics are summarized in **Table 2.** The sample had the following distribution in terms of age groups: 26.7% between 18–24, 28.3% between 25–34, 16.7% between 35–44, 11.7% between 45–54 and 16.7% between 55–64 years old. Of the entire cohort, 49 cases (81%) achieved at least 12 years of education. Based on the BMI values, 69.5% of participants were normal-weight and 23.7% were categorized as overweight.

Neuroimaging findings

The MRI findings for 56 volunteers are summarized in **Table 3**. The neuroradiological information was not available in 4 of the 60 cases: in one case due to technical problems, in a second because of claustrophobia, in a third out of safety concerns for a volunteer with an implant of undocumented material and in the fourth because the patient refused the MRI. Forty participants (71.4%) did not show any abnormality. Incidental findings of weak or no clinical relevance were reported in 16 cases (28.6%). Most of these were unspecific foci of T2/FLAIR signal abnormality (**Fig 1**). The Evans' Index (EI) represents a rough indicator of ventricular volume and was also computed. The EI is the ratio between the maximum width between the anterior

Table 2. Socio-demographic characteristics of the cohort (n = 60).

Sex (men/women: n, %)	38 (63.3) / 22 (36.7)
Age (years: mean ± SD, min, max)	36.2 ± 13.9 (18–64)
Education level (years: mean ± SD, min, max)	13.8 ± 3.6 (8–22)
Education level (higher level achieved)	
Primary education (n)	9
Secondary education (n)	2
High-school education or professional training (n)	23
Bachelor studies (n)	12
Postgraduate studies (n)	14
Body Mass Index[1] (kg/m^2: mean ± SD, min, max) (n = 59)	23.51 ± 2.97 (17.35–31.14)
Underweight (n)	4
Normal weight (n)	41
Overweight (n)	13
Obese (n)	1
HADS anxiety[1] (median, min, max) (n = 59)	5 (1–14)
Normal (n)	45
Borderline (n)	8
Elevated (n)	6
HADS depression[1] (median, min, max) (n = 59)	2 (0–8)
Normal (n)	57
Borderline (n)	2
Elevated (n)	0

HADS: Hospital Anxiety and Depression Scale; max: maximum score; min: minimum score.

[1] the BMI and the HADS scores are available for 59 participants.

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Table 3. Magnetic Resonance Imaging (MRI) findings (n = 56).

Nothing remarkable	40
Unspecific foci of T2/FLAIR signal abnormality	7
Punctiform white matter lesions (Fazekas 1)	3
Small venous angioma	3
Mild diffuse or focal atrophy	2
Microbleeding (possible cavernoma)	1

T2/FLAIR: T2-weighted or fluid attenuated inversion recovery (FLAIR) sequence

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horns of the lateral ventricles and the maximal internal diameter of skull, measured at the same level.[31] An EI value that exceeds 0.30 warrants further examination, and in this sample was 0.24 on average and varied between 0.20 and 0.29. All cases were thoroughly revised and lacked any medical condition that required excluding them from the group.

SCAT2 assessment and the reference interval calculation

The symptom profile, as reported by the volunteers, and the scores for all SCAT2 components are presented in **Table 4**. The participants reported a median of 3 symptoms (min-max: 0–11) (**Fig 2C**), with a severity score of 4 (min-max: 0–31) (**Fig 2D**). Encountering difficulties in the balance examination was frequent, as the M-BESS score had a median of 24, out of a maximum of 30 points. In the cognitive evaluation, the concentration and delayed memory scores varied the most, regardless of a high total SAC score (median: 27; min-max: 22–30). Orientation and immediate memory scores were perfect or nearly perfect in all cases.

The most frequently endorsed symptom was feeling difficulty in concentrating, with 41.7% of the sample reporting it on the day of the examination. In addition, more than 1 in 3

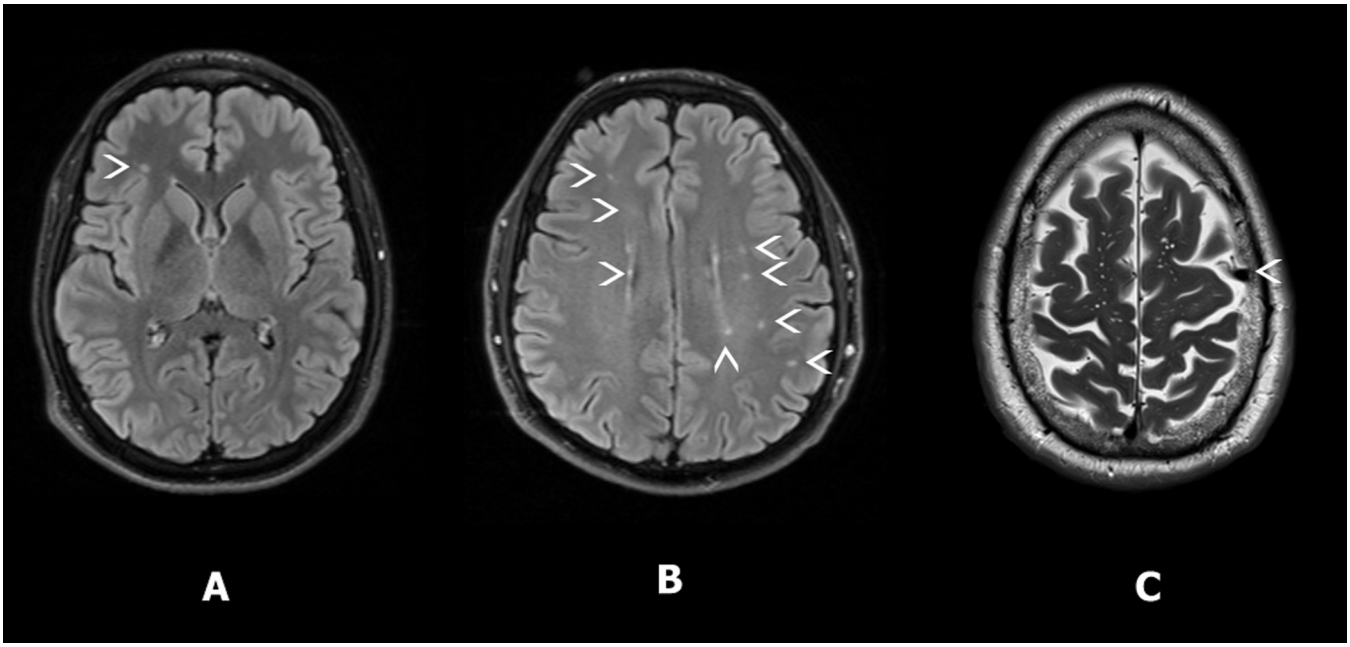

Fig 1. Examples of MRI incidental findings. Relevant details are marked with white arrowheads: A. Unspecific punctate lesion in a 21-year old male, in FLAIR. B. Multiple foci of white matter hyperintensity, corresponding to stage 1 on the Fazekas scale,[32] visible in the FLAIR scan in a 50-year old man. C. Venous angioma in a 58-year old man in a T2-weighted image.

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Table 4. SCAT2 scores and postconcussive-like symptom profile.

	median (min—max)
Number of symptoms	3 (0–11)
Severity score	4 (0–31)
Balance BESS	24 (14–30)
SAC scores	27 (22–30)
• Orientation	5 (4–5)
• Concentration	4 (2–5)
• Memory immediate	15 (13–15)
• Memory delayed	4 (0–5)
SCAT2 total	87 (71–97)
Most frequently reported symptoms	**n (%)**
• Difficulty concentrating	25 (41.7)
• Difficulty remembering	21 (35.0)
• Fatigue or low energy	21 (35.0)
• Nervous or Anxious	20 (33.3)
• Drowsiness	16 (26.7)
• Sadness	13 (21.7)
• Neck pain	12 (20.0)
• Trouble falling asleep	12 (20.0)
• Headache	11 (18.3)
Symptom clusters	
• Somatic	33 (55.0)
• Cognitive	34 (56.7)
• Emotional	26 (43.3)
• Sleep—fatigue	33 (55.0)

SCAT2: Sport Concussion Assessment Test, 2nd edition; BESS: Balance Error Scoring System; SAC: Standardized Assessment of Concussion

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participants reported feeling difficulty remembering, fatigue or low energy and feeling nervous or anxious. Sixteen volunteers (26.7%) endorsed more than 5 symptoms. Fifteen out of the 22 symptoms were endorsed by at least 10 percent of the sample. The exploration of the symptom profile at cluster level also indicated heterogeneity. Participants reported symptoms in the somatic, cognitive or sleep-fatigue clusters with similar frequencies (55%, 56.7% and 55% respectively), while 43.3% of the sample endorsed at least one emotional symptom.

The number of symptoms and the severity score distributions were significantly skewed to the right (Fig 2A and 2B) (Shapiro-Wilk's test for symptom number: $W = 0.92$, $p = 0.001$ and for the severity score: $W = 0.82$, $p < 0.001$). For both scores, the Horn's algorithm did not flag any outlier. Therefore, the entire cohort was included in the calculation of the RIs. As previously described, the distribution-free nonparametric reference intervals method was used, that calculates the 2.5 and the 97.5 percentiles. The upper interval boundary obtained was 10.5 for the symptom number, and 28.9 for the severity score.

Screening anxiety and depression

The HADS scores are presented in Table 2. Nearly the entire sample (96.7%) scored less than 8 points on the depression subscale, while only 2 cases were classified as borderline and no participant reported symptomatology that could have been considered clinically relevant. Although anxiety symptoms were more prevalent, 76.3% of participants scored in the normal

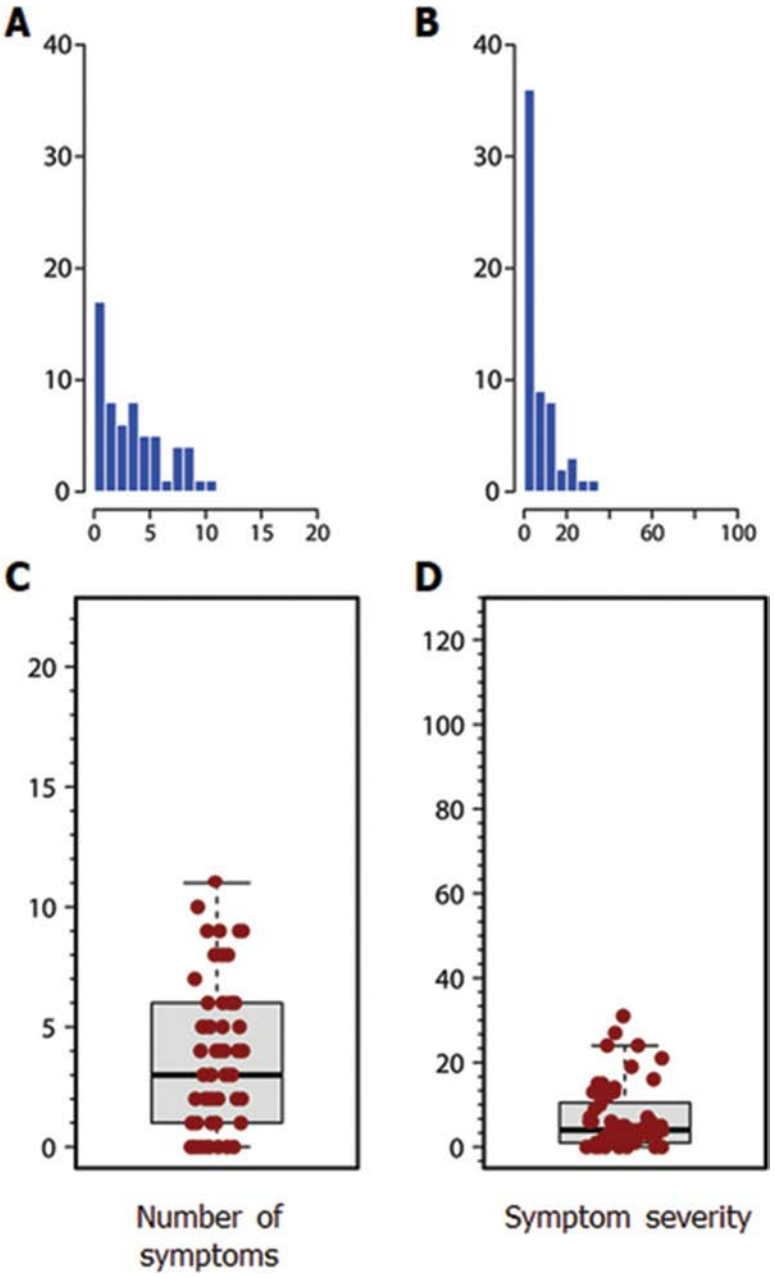

Fig 2. Distribution of the number of symptoms and the symptom severity score in the cohort. A. Histogram for the number of symptoms. B. Histogram for the symptom severity score C. Box-and-whiskers plot for the number of symptoms. D. Box-and-whiskers plot for the symptom severity score. The black lines inside the boxes are the median values for each group. The vertical size of the boxes is the interquartile range (IQR). The 'whiskers' represent the minimum and maximum values that do not exceed 1.5×IQR.

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range. Another 13.6% were borderline and the remaining 10% scored above the threshold indicative of a potential anxiety disorder.

Factors related to concussion-like symptom profile

In analyzing whether the symptom presentation differs by sex, women endorsed a median of 3.5 symptoms (min: 0, max: 9) and men a median of 3 symptoms (min:0, max: 11). These

differences were not statistically significant (Mann-Whitney U Test, W = 404, p = 0.83). The median severity score for men was 4 (min: 0, max: 31) and 5 for women (min: 0, max: 27). The differences in severity score were also not statistically significant (Mann-Whitney U Test, W = 415.5, p = 0.97).

To test if endorsed symptoms and their severity increased with age, Kendall's rank correlations were performed. The results showed no statistically significant association between age and endorsed symptoms (T = 0.14, $p = 0.13$), nor between age and the severity score (T = 0.16, $p = 0.07$).

Scatterplots were constructed to correlate the HADS total score, the anxiety subscale (HADS-A) and the depression subscale (HADS-D) with the number of endorsed symptoms and the severity score. At the predefined alfa level of 0.005, we did not find any association between the HADS total score and the endorsed symptoms (T = 0.19, $p = 0.037$) or the HADS total score and the severity score (T = 0.22, $p = 0.019$). We did not find any correlation when plotting the HADS-A score against the number of the endorsed symptoms (T = 0.18, $p = 0.068$) or the HADS-A score against the severity score (T = 0.21, $p = 0.029$). No association was found between HADS-D and the number of the endorsed symptoms (T = 0.19, $p = 0.057$) or the severity score (T = 0.18, $p = 0.067$).

Discussion

The incidence of post-concussion syndrome is widely heterogeneous, it ranges between 10–30% among different patient populations, and the diagnostic criteria are still inconsistent.[33, 34] Even the minimum number of symptoms and the time since injury required for diagnosis are a matter of debate, with the latter varying in different studies from 7 days to 3 months.[33, 35, 36] In the clinical evaluation and research studies of non-sport mTBI, inventories of self-reported symptoms are frequently used. The SCAT2 is the most widely-used structured tool in sports-related concussion assessment and it includes a 22-item self-report symptom scale.[15] Athletes usually have a preseason SCAT2 evaluation baseline, but in the clinical setting baseline measurements are lacking and therefore clinicians cannot perform the comparison of endorsed symptoms and their severity. It has been shown that 'healthy' individuals in the general population frequently report concussion-like symptoms. In order to identify symptomatology that manifests in the absence of head injury, most previous studies have targeted sports-related concussion and have described baseline evaluations of youth or collegiate athletes.[12, 21, 37] Some self-report tools, like the Rivermead Post-concussion Questionnaire,[38] tackle the absence of baseline evaluations by asking the respondent to distinguish between symptoms that have been present beforehand and others that have only become a problem following injury. Although this is a valuable approach, its reliability is limited by the 'good old days' bias, as patients with mTBI retrospectively report their preinjury status as better than the average person.[39] The aim of this study was to establish a population-based threshold for SCAT2 symptom profile indicators—number of symptoms, clusters and severity score—that could be used in multivariate analysis of non-sports mTBI cohorts with the purpose of discriminating patients with risk factors for presenting clinically-relevant PPCS that significantly affect patients' psychosocial functioning and of identifying different biomarkers that could predict them.

This study reports the reference intervals for concussion-like symptoms in a 'healthy' adult population between 18 and 65 years of age. Our data showed that non-concussed individuals frequently reported concussion-like symptoms. In this cohort, uninjured participants reported a median of 3 concussion-like symptoms and the upper reference interval was found at 10.5 symptoms, out of a total of 22. The median severity score was 4.9 points and 28.9 was the upper limit for the reference interval. It is worth noting that only 10 participants (16.7%) did

not endorse any symptom. This is in agreement with the findings of Iverson et al. who showed in high school athletes that 19% of boys and 28% of girls reported having a symptom burden resembling the diagnosis of post-concussion syndrome.[37]

Clinical research needs consensus in identifying goals that are clinically relevant and, in mTBI outcome assessment, this calls for agreement on a minimum set of symptoms and/or severity score. However, this is not yet the case. A recent survey on physician members of the American Society of Sports Medicine showed that 55% of the respondents considered that just '1 symptom' was enough for the diagnosis of post-concussion syndrome, while 17.6% of the participants required at least two symptoms.[33] The fact that our study yielded such elevated cut-offs scores in a healthy population makes low threshold used in sport injuries misleading when applied to a civilian population and warrants careful interpretation of the results of the SCAT2 inventory or any similar self-reported symptoms checklist in non-sports settings. In addition, establishing the clinical value of symptoms entails determining whether patients' symptoms are a consequence of the concussion or of other factors, especially when symptoms are reported weeks or months after injury. In this complex decision-making process, a criterion of any arbitrary number of symptoms that is to be used independently of their severity can only add to the confusion. We addressed this by conducting a population-based approach with a thorough characterization of participants that included MRI scanning and by using robust methods to flag outliers and establish an upper reference interval for self-reported symptoms. In addition to the clinical relevance of the present data, supervised machine learning models of mTBI could benefit from incorporating data-driven outcome thresholds. Supervised learning strategies—i.e. that aim to predict predefined output values from several input measures, such as logistic regression or random forests,[40] need to take into account the limitations of the traditional mTBI outcome scores, when symptoms commonly found in the general population are not considered.

It is worth mentioning that the MRI incidental findings in our cohort were not unexpected for a healthy sample, taking into account the high detection capability of 3 Tesla machines.[41] White matter hyperintensities (WMHI) were the most frequent finding in our sample (12.5%). In a cohort in which 41% of the participants were between 40 and 65 years old, the results are not remarkable. [42] As explained, the decision to not exclude participants with incidental findings from our study was made after careful considerations of their clinical background and known health status. In addition, other studies have called upon not excluding participants with WHMI from mTBI control groups[43], especially in DTI research. The exclusion of cases with common preinjury characteristics, like WHMI, that would not be excluded from a mTBI sample has potentially resulted in a systematic bias. In the present study, we have opted for a sample with increased representativity for the general adult population.

Looking further into the SCAT2 results, one factor that could explain the high incidence of concussion-like symptoms in this cohort is the method of assessment. As previously stated, the SCAT2 was chosen for its wide use in sport-related concussion literature and its suitability for clinical practice. However, various studies have shown that there is a statistically significant difference in symptom reporting when using different tools with open-ended questions, a simulated structured interview or a standardized checklist. The standardized checklists like the SCAT2 are the form of assessment that elicits the most symptoms, both in non-concussed students [44] and in patients with mTBI.[45] As previously stated, this highlights the need of consensus in mTBI medicine and that clinicians evaluating patients with mTBI need to be cautious when interpreting self-reported questionnaires.

Previous literature has tended to show that women endorse more symptoms than men, either as control volunteers or as athletes in preseason baseline evaluation and following concussion, and even that they are more likely to report different symptoms than men.[21]

However, in this cohort, there was no effect of gender and age on symptom presentation. Whether or not this is a robust pattern should be addressed by replication studies with a bigger sample size. It is also possible that the general population controls have different profiles than young college students and high-school athletes and, therefore, the observed differences in other studies could reflect the distinct composition of the studied samples.

Emotional distress is a frequent reaction to a traumatic event and it plays an important role during the recovery process. In mTBI research and in PPCS assessment, depression has always been considered a pivotal factor as it can trigger or exacerbate the manifestation of other post-concussion symptoms. Specifically, depressive and anxiety symptomatology is associated with fatigue, low energy and trouble sleeping and it is a better predictor for presenting cognitive complaints than objective neuropsychological functioning.[46] In this cohort, the levels of depression and anxiety were not linked to concussion-like symptom presentation. These results were produced by applying a stringent threshold for statistically significance ($p < 0.005$), arguably failing to report weak correlations between emotional distress and both symptom number and their severity, and between anxiety and symptom severity (Tau between 0.19 and 0.22, $p < 0.05$, Kendall's). Regarding depression, the results are explained by the absence of participants with evident depressive symptomatology. As few moderate and no high scores were obtained in the HADS-D subscale, the variation in the profile of concussion-like symptoms could not be linked to depression.

Study limitations

Several limitations to this study are acknowledged. The participants were selected from a single tertiary hospital and this may limit generalizability. In addition, the enrolment of next-of-kin of patients admitted to a neurosurgical department could result in a cohort with higher stress levels than community population. All preexisting health conditions were self-reported and were not verified. In addition, because participants were selected with predefined demographic characteristics, this cohort was not representative of the Spanish population in terms of age and gender distribution. It disproportionately includes more men, and more participants between 18–24 years of age and less participants between 25–54. Furthermore, the sample size ($n = 60$) is relatively small, in comparison with other multi-center studies that present normative data for concussion assessment. Future studies should be directed at improving normative data in specific subgroups of civilian population by increasing the sample size and including elders. As explained previously, clinical anxiety and depression symptoms can modulate the presentation and resolution of mTBI symptomatology. Regardless general anxiety or depression levels, because our cohort includes companions and relatives of patients, it is possible that they pay more attention to their own body and mental state and could even exhibit a different pattern of symptoms, as biased by this exposure to health issues. Future studies could address how concerns about one's health (in relation with specific stressors like the illness of a relative) relate to the manifestation of symptoms in the general healthy population.

In addition, falls in people over 70 years old are the most frequent scenario of mTBI, but the particularities of injury mechanism, comorbidities and preinjury treatment in this cohort complicate its inclusion in a general adult mTBI sample. Therefore, assessing the necessity of establishing different normative scores for these distinct mTBI populations would be a valuable advancement of the present study.

Conclusions

PPCS are a cause on ongoing disability and distress for affected patients and a source of high healthcare costs. Further studies directed toward identifying individuals who are at risk for

developing PPCS after mTBI are important to both patients and healthcare professionals. Prognosis models should not rely on the use of arbitrary cut-off scores for symptom-related variables, because they currently fail to reflect the frequent presentation of similar symptoms in the absence of prior head injury. Our results suggest that for a better refinement of the patient selection, the outcome variables should be redefined to take into consideration that some concussion-like symptoms can be endorsed by healthy individuals.

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Author Contributions

Conceptualization: Andreea Rădoi, Maria A. Poca, Juan Sahuquillo.

Data curation: Andreea Rădoi.

Formal analysis: Andreea Rădoi, Maria A. Poca, Juan Sahuquillo.

Funding acquisition: Maria A. Poca.

Investigation: Andreea Rădoi, Darío Gándara, Lidia Castro, Mauricio Cevallos, Maria E. Pacios.

Methodology: Andreea Rădoi, Juan Sahuquillo.

Project administration: Andreea Rădoi, Maria A. Poca, Darío Gándara, Mauricio Cevallos, Juan Sahuquillo.

Supervision: Andreea Rădoi, Maria A. Poca, Juan Sahuquillo.

Visualization: Juan Sahuquillo.

Writing – original draft: Andreea Rădoi, Juan Sahuquillo.

Writing – review & editing: Andreea Rădoi, Maria A. Poca, Darío Gándara, Lidia Castro, Mauricio Cevallos, Maria E. Pacios, Juan Sahuquillo.

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V. Discussion

In this section, the main results of the studies that were carried out for this thesis will be outlined and reviewed in the context of the most recent literature available. The relevance of these results will be scrutinized from the point of view of clinical applicability and future research opportunities will be explored. Limitations to the studies will be discussed in detail.

In brief, the results of the first study showed that patients can present with symptoms that persist for days following a concussion, with reasonable implications for their return to previous activities and quality of life. In addition to subjective post-concussion symptoms, these patients present with cognitive deficits which are detectable with specific assessment tools in the first 1–2 weeks following the mTBI. Lastly, in some patients with an early normal CT scan, the MRI exploration identified microhemorrhages and lesions compatible with DAI. There are further issues to be addressed concerning the selection process, the mTBI and concussion criteria and the generalizability of this study. These considerations are presented foremost, as they should be kept in mind throughout the discussion of the remaining results of the study.

In light of significant discrepancies between methods of poor outcome identification in mTBI research, in the second study, a standardized concussion assessment screening test was used to identify the prevalence of significant concussion-like symptoms in the healthy population with no history of head trauma. As a result, reference intervals for concussion-like symptoms in a "healthy" adult population between 18 and 65 years of age were reported. In this cohort, non-concussed participants reported a median of 3 concussion-like symptoms and the upper reference interval was found at 10.5 symptoms, out of a total of 22. The median severity score was 4.9 points and 28.9 was the upper limit for the reference interval. This score is significantly higher than traditional cut-off scores, and the case was made that future prognosis studies in mTBI outcome, that use strategies such as logistic regression or random forests, would benefit from taking into account the limitations of the traditional mTBI outcome scores, when symptoms commonly found in the general population are not considered.

1. Concerning the recruitment process and the mTBI–concussion diagnosis conundrum

One of the most striking findings from the first study was the very small number of patients included after the recruitment selection, considering that 1,144 patients were screened in the lapse of a year. As presented in detail previously, only 75 (6.5%) participants fulfilled all inclusion and none of the exclusion criteria and eventually the study examinations were performed on an even smaller number (41, 3.6%), due to the rejection to participate in 34 cases. First and foremost, this finding highlights the heterogeneity of mTBI population and acknowledges that the investigation conducted afterwards, focused on the presentation and outcome of these patients, may not be pertinent for more than 90% of the patients attending an emergency department with similar characteristics to our center. Although this result is not unique to our work (Isokuortti et al., 2015; Luoto, Tenovuo, et al., 2013), too frequently the authors of papers on clinical samples do not stress enough the effect that such a conservative selection process can have on the interpretation and applicability of their own results.

The aim of this project was to explore a subgroup of mTBI patients with a "pure concussion", working age adults in good general health with as few aggravating factors for presenting PPCS as possible. Considering the high number of mTBI patients attending our center, this approach seemed reasonable. Patients older than 65 years of age were excluded because of different mechanisms of injury and higher likelihood of comorbidities. However, after establishing that more than 55% of all attendees were above that age threshold, researchers should not drive their attention away from the particularities of concussion in this group, due to the profound impact that an improvement in the clinical management of this subgroup could have.

A systematic review about the characteristics of the patients enrolled in studies on the value of blood BMs detected after mTBI for the prediction of PPCS found that the most frequent exclusion criteria were neurological disorders, in 55.6% of the studies, and psychiatric disorders and trauma to another body region, both applied in 47.2% of the studies (Mercier et al., 2017). These are sensible exclusion criteria, but in our sample 28.3% patients were excluded due to neurological or psychiatric history or previous TBI (104 of 367 patients screened for this) and another 14.7% due to alcohol or substance abuse disorder. Therefore, the generalizability of the results of our studies remains significantly limited, as in approximately one half of the published works on BMs in relationship with PPCS.

The problem of recruitment in mTBI studies is strongly tied to the mTBI and concussion diagnosis criteria. We have excluded participants with a GCS score of 13, which in theory contravenes the consensus classification. However, for decades there have been arguments for excluding these patients from the series with mTBI, because of worse prognosis and disparate risk of intracranial abnormalities visible on CT scan. Moreover, a recent multinational European survey showed that 38% of the respondent centers classified patients with a GCS score of 13 as moderate TBI (Foks et al., 2017). Therefore, more than a third of respondents are applying the GCS

classification system in the same way as the present studies. Moreover, patients with a GCS score of 13 were excluded in at least 20% of the studies from a systematic review examining the prognostic value of BMs for PPCS (Mercier et al., 2017).

This is linked to another exclusion criteria of our study: normal CT findings. Any objective finding of brain injury following mTBI changes the treatment indication (as it is far more likely to include a routine follow-up visit), the conversation about the traumatic event and the patient's expectations of a complete, uncomplicated recovery. Although in a vast majority of cases concussion is associated with physiological disturbance and not with structural injury, the search for a biological basis of the concussion symptoms is potentially biased by excluding cases with lesions visible on a CT scan despite displaying an identical manifestation. As concussion cohorts are being examined with neuroimaging tools superior to the CT scan, the evidence for functional and structural brain injury markers will boost. The restriction of a concussion diagnosis to cases with negative CT results was driven by identifying cases with purely physiological disturbance, but updating the definition of concussion in a manner that continues to exclude novel biomarkers of structural injury is unsustainable (Bigler et al., 2016).

This study addressed the presentation characteristics, brain injury burden and outcome of a subgroup of mTBI patients (with GCS scores of 14 or 15) that lacked any driver for clinical decisions as strong as a GCS of 13. Another important exclusion criterion was related to mTBI without concussion. This was noted in 55% of cases assessed for this: 92 of the 167 patients between 18 and 65 years old, who had no relevant medical history and arrived at the center within 24 hours after they hit their head were not considered concussed. Concussion was diagnosed in the presence of one of the following signs or symptoms: LOC, PTA, seizures, vomits or intense PCS. All cases of mTBI with recovered LOC, PTA and GCS of 14 would automatically be eligible, under all mTBI – concussion criteria. Very few patients presented with seizures (< 1%) and all would have fulfilled inclusion criteria regardless.

The reason motivating the recruitment of patients with GCS of 15, no LOC, PTA, nor seizures only in the presence of indicators of physiological disruption (as vomiting or severe symptoms at the moment of the ED evaluation) was to exclude head injury with no sign of concussion. However, the rationale that a concussion must be associated with a display of symptoms that induce significant discomfort in the first hours after the mTBI is questionable. In theory, this illustrates a problematic circularity, as selecting the mTBI population based on their symptoms would parallel using the outcome of interest as a diagnostic element. However, this is the only strategy available as no clinical factor, biological marker or combination of both has been found sensitive and specific enough to allow for a reliable diagnostic of concussion–mTBI (Lees-Haley et al., 2003). This methodologic issue is very relevant for studies that select the population based on presenting PPCS, either for transversal investigations or for retrospective analysis, but it is only in part applicable to studies as ours were the recruitment is made on very early symptoms (< 24 h) and involves a long-term follow-up.

In relation to the same criterion, some cases that were considered "trivial trauma" could have been recruited in other similar studies. Subconcussive blows are especially relevant in the study of repetitive injury but inquiring into the physiopathology of single subconcussive blows should not be dismissed as superfluous. For example, in a remarkable study, head injury patients that did not fulfil mTBI criteria (labelled HIBRID: "Head Injury Brain Injury Debatable") had a higher prevalence of incomplete functional recovery than orthopedic patients (Korley et al., 2016). Although the risk of presenting poor outcome was lower for the HIBRID group than for patients with mTBI that fulfilled the ACRM criteria, the results suggest that the indicators in use for estimating the presence of brain injury following mTBI are poor. In addition, the CT scan results reported traumatic brain lesions in 9 HIBRID patients (10%), undoubtably indicating that brain injury is not anecdotical in these patients (Korley et al., 2016). The authors argue that, in many cases injury involves the frontal lobes or other neocortical areas and the signs or symptoms that are currently necessary for mTBI identification are lacking.

The same can be put forth concerning concussion. As it appears that the same pathophysiological processes associated with concussion also take place in cases lacking strong clinical indicators, the clinical entity is too inconsistent. The consideration that concussion is a particular form of a lesser severity of mTBI, and that head injuries that do not fulfil current mTBI or concussion criteria are benign is faulted. As exposed by Breton Asken, the physiologic disturbance of a concussive event needs its own definition and must stop relying solely on the presence or absence of symptoms (Asken, 2019). Until then, studies are going to unavoidably enter the same pitfalls.

2. The control group

One of the qualities of these studies is the inclusion of a control group. Many studies on TBI prognosis rely solely on the data provided from the group of patients, ignoring the valuable information that can arise from the inclusion of a comparison group of healthy participants without head injury. Particularly in mTBI, this topic has been problematic; it can be argued that the association between subjective complaints and a traumatic etiology is not as straightforward when taking into considerations the incidence of the same profile of grievances with no history of mTBI.

Before discussing the results of the concussion-specific evaluations that were performed in the control group, several issues must be reviewed. First, all participants in the control group were scheduled for an MRI exploration and forty participants (71.4%) did not show any abnormality. In seven cases (12.5%) the incidental findings were WMHI, while another 3 cases presented with punctiform white matter lesions. For a cohort that included participants up to 65 years old, these findings are not remarkable (Evans, 2017). In a study that included a group of 30 adults with similar criteria (no history of neurologic, psychiatric or brain injury), 6 cases (20%) were excluded due to

clinically relevant incidental findings (Wäljas et al., 2014). Two participants (6.7%) had few non-specific WMHI and, just as in our study, were not excluded from the control group.

Another issue concerns the selection of "healthy" community dwellers and not patients with orthopedic injuries (OI). Some researchers argue that patients with mTBI share with the ones with OI injury-related experiences, particularly pain, fatigue and inconveniences related to receiving medical attention. However, results of studies with children, adolescents and adults have questioned the added value of an OI group, in addition to a control group comprising healthy, typically developing members (Beauchamp, Landry-Roy, Gravel, Beaudoin, & Bernier, 2016; Mathias, Dennington, Bowden, & Bigler, 2013; Schretlen & Shapiro, 2003). Furthermore, a carefully selected control group with community dwellers has proved essential for identifying poor cognitive recovery following mTBI, whereas the cognitive outcome was similar between the mTBI and the OI groups (Rabinowitz et al., 2015).

3. The use of SCAT2 in civilian assessment of mTBI

One of the first goals of this project was to improve the characterization of patients following mTBI at their earliest medical assessment, which frequently takes place in the ED. Sociodemographic characteristics, the GCS score, LOC, PTA and other simplistic injury-related descriptors are not enough to guide outcome prognosis. In other to be able to predict protracted post-concussion recovery, an improved systematic assessment of their clinical presentation was needed. It is undeniable that the most beneficial prognostic methods in mTBI should be available during the ED visit, since patients are not routinely scheduled for follow-up (Lingsma & Cnossen, 2017).

In 2012, when the protocol was designed, there was no published article on the use of SCAT2 in civilian mTBI. Since then, articles testifying on the suitability of the SCAT tool to the clinical examination, unrelated to sports concussion, have been published worldwide (Luoto, Silverberg, et al., 2013; Nelson et al., 2017; Sussman et al., 2017). Furthermore, SCAT3 has even been used in the systematic assessment of a small series of hospitalized TBI, that included mild cases with abnormal CT findings (Sargeant et al., 2018).

In our cohort, as expected, patients display on average a higher number of symptoms after a concussion than non-concussed individuals, both during the first 24 hours post-injury and at 1–2 weeks. Likewise, the severity of their symptoms is significantly higher than the concussion-like symptoms reported by the control group. On the other hand, the systematic assessment of early concussion symptoms is fundamentally relevant for outcome (Cassidy et al., 2014). In a systematic review of predictors of clinical recovery following SRC, 87.5% of the examined studies found a significant association between a greater acute symptom burden and a delayed recovery (Iverson et al., 2017), leading the authors to conclude that the initial symptom presentation is the strongest

and most consistent predictor. The early symptoms have also been found to independently predict recovery time in military samples (Kennedy et al., 2012) and in clinical civilian cohorts (Luoto, Silverberg, et al., 2013; Nelson et al., 2017). In our sample, this association was analyzed in the Supplementary Material, Sections B, C and D.

Before being included in the SCAT series, the cognitive global index, SAC, has been extensively used a stand-alone tool for examining concussion in sports-related research and in military studies, as part of the Military Acute Concussion Examination (MACE) (Kennedy et al., 2012). It is considered a sensitive tool in the first 48h after a concussion, and it has found to correlate with white matter integrity as assessed by DTI, although not with blood BM levels of GFAP and UCH-L1 (Kou et al., 2013).

In the sample included in the first study of this thesis, in the early 24 hours post-injury, the median SAC score was 25 (min 20, max 30), which is comparable with other reports. Therefore, half of this heterogenous adult sample achieved scores below the normative threshold used in SRC, which is 25 (McCrea et al., 1998). However, the SRC results cannot be directly extended to a civilian adult population, primarily due to a longer time between injury and examination than in SRC studies (were the examination is performed immediately on the sideline), and due to significantly older participants. SAC was also used in the evaluation of a convenience ED-based sample with concussion and the average score was 21 (SD 5.4) (Naunheim, Matero, & Fucetola, 2008). In that study, participants were evaluated three times during their stay of 6 hours in the ED and their scores gradually increased, which is compatible with an evolution in the acute stage post-concussion. Notwithstanding, the authors did not report time since injury at the first ED evaluation, so it is difficult to compare their results with ours. In another more recent study, that included an extensive description of the sample, Luoto et al. found that patients obtained on average a SAC score of 25.1 (SD 1.8) in the first 24h after an mTBI; the SAC score reasonably discriminated between mTBI and OI patients and was associated with MRI brain lesions. However, they also reported that the SAC score minimally improved the classification achieved by the number of symptoms alone (Luoto, Silverberg, et al., 2013). All in all, it can be concluded that concussion was associated with a noticeable decline in the cognitive global index, in a significant number of patients from our cohort and that said cognitive decline improved over time, as expected. In comparison to the other ED-based studies, the one included in this thesis is the first one that examined only patients with normal CT findings.

The modified balance error scoring system (mBESS) is a section that deserves further consideration. It has been designed as a tool sensitive to brain injury (on the premise of normal vestibular function), and has been consistently considered useful in SRC assessment and even in severity stratification (Garcia et al., 2019). However, there are studies that show surprisingly poor results in the general population and cast doubt about its suitability for civilian mTBI assessment (Inness et al., 2019; Luoto, Silverberg, et al., 2013). In the earliest examination following mTBI or in the presence of other neurological conditions (which were excluded from this study but are assessed in all medical centers), patients can present a standing difficulty. One study found that as

much as 46.9% of cases could not complete the mBESS examination due to polytrauma or symptom severity (Nelson et al., 2017). They also showed that the inability to complete the balance items was a predictor for a protracted recovery while the score in itself, for the patients who completed the task, was not (idem). In our complete sample, 18 (22.5%) could not attempt the task and another 4 (5%) were scored 0 out of 30 possible points (Supplementary Material, Section C). In effect, these findings mainly support the separate use of the SCAT subscores and highlight the lack of utility of the global SCAT2 score, given the frequent cases in which it cannot be calculated. Future editions of the SCAT or a clinical equivalent tool would probably benefit from the incorporation of a codification system for the patients not able to complete the mBESS.

4. Beyond normal CT findings in concussion

The results of the second article were obtained in a subgroup of 20 patients that underwent MRI scanning in the first 2 weeks following mTBI with concussion. Despite having presented with no abnormalities visible on CT scan, 2 cases (10%) had injuries compatible with DAI. Another 5 cases (25%) presented abnormalities which had not been visible on the CT scan, particularly WMHI. Because their etiology was uncertain, they were not reported as related to the traumatic event. The MRI results on the entire cohort of patients revealed microhemorrhages in a total of 4 out of 49 mTBI cases (8.2%) and WMHI were reported in 7 cases (14.3%) (unpublished data).

As pointed out previously, WMHI are frequent findings in participants with no history of head injury. However, WMHI have been systematically associated with an increased risk of stroke, dementia and death (Debette & Markus, 2010) Due to their lack of specificity for injury, a careful clinical interpretation is warranted although their functional effect in the concussed brain remains unclear. Brain abnormalities than are common and silent in a healthy or resilient brain might induce or enhance neuropsychological dysfunctions in the context of injury. This hypothesis has been explored in a study with mTBI in the military population (Tate et al., 2017). The authors found that the prevalence of WMHI did not differ between mTBI, OI and PTSD groups. However, examining the cognitive status within the mTBI group, the participants with WMHI had a statistically significant distinct level of executive functioning (specifically, worse working memory). On the other hand, participants in the OI and PTDS groups did not differ according to the presence or absence of WMHI results. At the same time, the same cohorts were examined with SWI, and the sequence proved to be sensible and specific to lesions in the mTBI group (22% in mTBI versus 1% in OI, and 0% in PTSD). However, the microbleeds, as detected by SWI, were not correlated with the neuropsychological status in that mTBI cohort.

Microhemorrhages were not associated with early post-concussion complaints, in a cohort of 54 patients with mTBI and normal CT scan findings (van der Horn, de Haan, Spikman, de Groot, & van der Naalt, 2017). On the other hand, the authors reported a considerably higher prevalence than in our findings (28% versus 8.2%), in a sample that appears clinically similar.

Notwithstanding, other studies argue that microbleeds are not that frequent in mTBI, as Toth et al. who found none in a smaller sample of 14 patients (Toth et al., 2013).

The potential for added value of the MRI findings in cases with a false-negative CT scan but persistent symptomatology should not be minimized. Complaints that previously would have been considered unexpected or psychogenic can be, at least partly, explained by brain injury. Having a clear explanation for perceived deficits or any subjective discomfort can have a significant contribution to the patient's realistic self-narrative and expectations post-injury. In addition, the extension, severity and recovery of the lesions can be monitored if needed, ultimately giving the opportunity for an improved individualized treatment.

Our findings add to the body of evidence that CT-negative results should be interpreted with caution in the mTBI population, as injury markers visible on MRI are not exceptional. We acknowledge the reduced prevalence of injuries limited the analysis in our sample. Potential stratifications of mTBI severity and the relationship between MRI findings and outcome have not been tested in this study. However, after a careful review of the literature, the functional role of WHMI and the clinical relevance of abnormalities visible on SWI currently remain unclear in unselected mTBI population.

5. Neuropsychological deficits in the first 2 weeks following mTBI

In the first article, the neuropsychological assessment performed at 1–2 weeks following mTBI showed statistically significant lower scores on several cognitive functions. In this sample, verbal learning, immediate and delayed memory were the most significantly altered, with moderate effect sizes. Visual learning and memory were also found reasonably sensitive to dysfunction after mTBI. Several variables concerning working memory and inhibition were suggestive of an alteration of executive functioning. The cognitive alterations were frequent and severe enough to be detected at group level, with traditional neuropsychological tools.

These results are in agreement with multiple studies that report a decline in cognitive status in the first 2 weeks following concussion (Carroll et al., 2014). Particularly, memory was deemed the most altered cognitive domain following TBI of all severities, in studies of moderate-severe patients (Levin, Goldstein, High, & Eisenberg, 1988; Scheid, Walther, Guthke, Preul, & von Cramon, 2006) and in mTBI (Dikmen, Machamer, & Temkin, 2017; L'Ecuyer-Giguère et al., 2018; Yallampalli et al., 2013). Post-traumatic declarative memory deficits are dependent not only on damage to the hippocampus and fornix, but also to the corpus callosum, the uncinate fasciculus and the cingulum (Niogi et al., 2008; Palacios et al., 2011).

Traditionally, attention was used to be considered a very sensible indicator of dysfunction in mTBI outcome (Cicerone & Azulay, 2002; Reitan & Wolfson, 2000). On the one hand, in this sample we found no differences with the control group in several variables tapping attention

(TMT-A, Omission number, first trial of visual memory). On the other hand, the decrease of score in Digits, although with a small effect size ($r = 0.27$), would support that, although that difference appears to be mostly explained by an effect in working memory, i.e. detected by the backwards component of Digits. Evidence in fMRI studies support that working memory deficits after mild and moderate TBI are a result of catecholaminergic dysregulation (McAllister, Flashman, McDonald, & Saykin, 2006).

In a subgroup of patients with mTBI and normal CT-scan findings, Iverson et al. found significantly better neuropsychological scores that in a similar mTBI group with CT-positive injuries. The highest effect sizes were observed in cognitive flexibility (as evaluated with TMT-B), and visual and verbal memory. Although they did not use any control group, their results would suggest that the mnesic functions are the most sensitive following mTBI, as in our study. In return, we did not find a statistically significant difference between groups on TMT-B scores.

In another ED-based study, in comparison with a control group of age-, sex-, and education-matched healthy participants, patients with mTBI presented verbal learning deficits and speed of information processing deficits at 1 week post-injury (Heitger et al., 2006). Again, this study confirms memory as a vulnerable cognitive function in these patients. However, their report on objective reduction in speed processing was not confirmed by our study. In out cohort, multiple variables that covered reaction time in simple or complex attention tasks failed to detect any objective decline in speed of processing, in comparison with the control scores. Cognitive processing speed is highly dependent on white matter integrity, which is especially vulnerable following TBI. Traditional neuropsychological tools that tap processing speed are relying on behavioral metrics, and their performance is measured in seconds and controlled by the examiner with a stopwatch. After concussion, the reaction time can be delayed by approximately 100-200 milliseconds (Bigler, 2013). In our study, we have tried to overcome this limitation by incorporating a computerized test (CPT v5.2), but the results in the mTBI group did not differ from the control levels.

Failure to find deficits in the verbal fluency skills has been reported previously, although the examinations were performed at slightly more than 2 weeks since injury (Leininger, Gramling, Farrell, Kreutzer, & Peck, 1990; Mathias, Beall, & Bigler, 2004). Other authors have found that fluency assessment was the most sensitive in reflecting mTBI-induced neuropsychological dysfunctions (Belanger et al., 2005). Apart for methodological discrepancies (for example, considering verbal with or without visual fluency), these discordant results could be explained by distinct pattern of injury between the cohorts. Although it is considered one of the most sensitive tests to frontal dysfunction, verbal fluency is not specific to frontal lobe damage. Poor scores on verbal fluency tasks could be explained by inefficiency of lexical access, but also a loss of cognitive speed, among others (Alvarez & Emory, 2006; Jurado & Rosselli, 2007).

Our results show that early post-concussion memory and executive difficulties are reflected by objective assessment in a significant number of patients. It is worth noting that this does not

imply that all patients experience significant cognitive decline in the first days and weeks following concussion. Various studies found no differences between their cohorts with mTBI and controls with the neuropsychological battery of their choice (Mayer et al., 2010; Wu et al., 2010). In addition, functional MRI studies have showed that in some cases, despite normal neuropsychological results, the brain expenditure of energy is increased, as an abnormal allocation of resources takes place to sustain cognitive functioning at normal parameters (McAllister et al., 2001). This could explain the cognitive complaints and the fatigue frequently reported post-concussion, regardless of neuropsychological scores. Neuropsychologically established deficits, as the ones identified in our study, are proof of the complex brain dysfunctions that appear in concussion. Neuropsychological tools are useful in characterizing post-concussion status, but they should be included as part of a broader assessment battery.

6. PPCS and poor outcome following mTBI

The results of our study show that the raw number and severity of post-concussion symptoms is consistently higher in patients with mTBI than community-based healthy control participants, on the day-of-injury and in the first two weeks.

The clinical decision-making about what constitutes a symptomatic profile that requires treatment is driven by a contextual assessment, particularly including the presence or absence of perceived worsening following injury, the presence or absence of other factors that have taken place since injury that could explain the symptoms better, and any daily life disruption they might cause. Recently, the ICoMP recommended that the term "post-concussion syndrome" be replaced with posttraumatic symptoms, because they are common to all injuries (Cassidy et al., 2014). We agree with the stringent need of addressing the lack of specificity of concussion symptoms, but we consider there is too much evidence tilting the scales towards the involvement of brain injury to resolve the conversation by altogether eliminating the reference to the "brain commotion". After a careful review of the literature, we felt the conversation about clinically relevant post-concussion symptoms would benefit from a different approach.

6.1. The need for a different criterion

The controversy surrounding a clinically relevant poor outcome following concussion hampers with any attempt to elucidate early indicators of increased severity or likelihood of complicated recovery.

> *"In most cases, no significant difference was found between the symptoms reported by the head injured group and the uninjured group. These results*

demonstrate the importance of establishing base rates of symptoms in non-clinical populations prior to drawing conclusions about symptoms in clinical populations."

(Gouvier, Uddo-Crane, & Brown, 1988)

Even though the problem had been exposed decades ago, few current studies tackle it appropriately. In the second article, we reported the reference intervals for concussion-like symptoms in an adult cohort, between 18 and 65 years of age, that was considered healthy. Participants with no history of TBI or concussion reported a median of 3 concussion-like symptoms. The upper limit of the reference interval was found at 10.5 symptoms, out of a total of 22. Furthermore, only 10 participants (16.7%) did not endorse any symptom.

It is worth adding that distinguishing between patients with what we defined as clinically relevant PPCS does not eliminate the need to care evaluate, treat and assist patients in their recovery of any symptom. We are arguing that identifying a subgroup of concussion patients which is clinically and "statistically" distinct from individuals with no history of head injury is the first step in achieving robust biomarkers that are neuropathologically associated with the manifestation of concussion. In this way, in a second step, biomarkers that are reliably linked with the symptomatology of concussion can be examined in samples with clinically uncertain PPCS. Even if the biomarkers identified will not prove equally useful for both subgroups of patients with concussion, the results of this approach could, at a bare minimum, confidently steer the investigation into alternative causes or enabling mechanisms for chronification.

6.2. Gender and emotional distress in PPCS

Among the most frequently studied factors in relation with displaying PPCS are gender and depressive symptomatology. In the first study, the questionnaire used to screen for anxiety and depression symptoms did not establish any statistically significant difference between the two groups. Although this suggests that emotional distress is not a variable that explains the differences reported in number or severity of symptoms between the two groups, we did not include an analysis to explore this specifically on this subsample. However, this hypothesis was examined, and the results of this analysis are included in the Supplementary Material, Section C.

Regarding the display of concussion-like symptoms in the healthy controls, we reported no effect of gender, age and emotional distress on symptom presentation. Due to the stringent level chosen for statistical significance ($p < 0.005$), several correlations involving the HADS scores did not reach the threshold although the corresponding p was < 0.05. At best, general emotional distress was associated both with symptom number and their severity, and anxiety particularly with symptom severity, but the strength of all those correlations was very weak (Kendall's τ between 0.19 and 0.22,). It appears that the variation of scores in this screening test, mostly within normal ranges, is not associated with concussion-like symptoms. However, due to the complex relation between premorbid emotional regulation impairment, post-traumatic affective symptoms and

general post-traumatic recovery, and the mixed results, future studies should address this in samples with affective disorders.

Although the relationship between sex and post-concussion recovery has been studied extensively, the results are mixed, as explained in this systematic review by Iverson et al. (2017). Multiple studies have found that girls and women, on average, report more and more intense symptoms and take longer to recover. Psychological factors are not the only ones that play a role in this, as previously thought, and differences in neck strength and injury biomechanics have been shown to independently explain the distinct symptom presentation. However, Iverson et al. reported more than 25 studies that have not found the female sex as a risk factor for protracted recovery following SRC.

6.3. Other methodological issues in PPCS assessment

Another issue concerning symptomatology assessment in our studies concerns the SCAT2 checklist. Rivermead Post-concussion Questionnaire (RPQ) (King, Crawford, Wenden, Moss, & Wade, 1995) has been gradually establishing as the gold standard in PCS assessment (Ngwenya et al., 2018; Wilde et al., 2010). RPQ assesses a list of 16 symptoms that are all but two contained by the SCAT symptom checklist. RPQ includes "double vision" in addition to "blurred vision" –which exists in SCAT–and "feeling frustrated or impatient", in addition to the similar items "being irritable, easily angered" and "restlessness" that have their SCAT equivalents in "irritability" and "nervous or anxious". However, in RPQ the patient compares its state over the past 24 hours with how he/she felt preinjury and scores each item on a 5-point Likert scale: 0 (not experienced at all), 1 (no more of a problem), 2 (a mild problem), 3 (a moderate problem) and 4 (a severe problem). One of the strengths of the RPQ is the self-reported comparison to baseline, but it requires the patient to evaluate its state over a reasonable amount of time since the mTBI. We have found no study were RPQ was applied in the first 24 hours following mTBI. As finding factors that would be available to clinicians for inclusion in a prognostic model during ED assessment was one the most important goals of the global project and RPQ's application is not suitable in the first 24 hours after injury, we did not include it in our protocol. We did not include it in the follow-up battery either because of its overlapping with SCAT. It has been established that the probability of endorsement symptoms on checklist depends on the form of eliciting the response, and practically doubling the number of symptoms, in parallel forms with different timeframes, would have undoubtably induced a bias in our study.

Notably, after the publication of our study, a very large multi-national study (N = 11,759) reported the prevalence of concussion-like symptoms in the general population after performing an internet-based assessment using RPQ (Voormolen et al., 2019). Half of the sample reported fatigue (49.9%) and almost half (45.1%) of the respondents were classified as having PCS after endorsing as problematic 3 or more symptoms (individual score ≥ 2). They also reported a median score of 8 (IQR 0–20), but, due to the structure of the RPQ, that score is not akin to the raw number

of concussion-like symptoms, which they did not report. Neither did they report the number of asymptomatic participants.

Furthermore, the lack of specificity of PPCS should not deter from acknowledging them as inexorably linked to the traumatic incident. Once more, our interpretation continues a line of expert opinions that appear to have been forgotten in recent years, despite not having been rebutted. Particularly, we consider important to assess late-term manifestation following a concussion as part of the same neuropathological process.

> *"If one accepts this definition of concussion, which is essentially a clinical rather than a pathological definition, then one would expect the symptoms that characterize it to be spoken of as concussion symptoms rather than postconcussion symptoms. The pain of fractured ribs may continue for some weeks, yet nobody refers to such pain as 'post rib fracture' pain."*
>
> (Rutherford, 1989, p. 218)

7. Additional limitations

The conclusions of these studies must be tempered by limitations of the data set. As exposed previously, several characteristics of the enrolment of the control group may limit generalizability and bias the endorsement we described of the concussion-like symptoms.

In addition, the neuropsychological analysis was limited by the sample size and included 34 patients and 28 healthy participants. Furthermore, the statistical significance level did not take into account the error associated with multiple comparisons. Another question that remained unanswered by the results of this study is the long-term outcome of these patients, as the analysis did not depict the follow-up later than 2 weeks post-injury. These issues were covered in the Supplementary Material, Section C where data from 70 patients at 1–2 weeks after mTBI were analyzed and compared with a control group of 60 participants. Moreover, the results on the 3-months outcome of this cohort are presented. For this purpose, the neuropsychological data were analyzed with an approach that aimed at reducing the type I error in comparison statistics.

Various limitations to the analysis of the MRI data of patients with mTBI are acknowledged. Due to the reduced absolute prevalence of microhemorrhages visible on SWI, the burden of injury could not be assessed through a semiquantitative scale nor statistically associated with outcome. Likewise, the group's presentation and outcome were not analyzed depending on the presence or absence of WMHI.

Future studies should be directed at improving normative data in specific subgroups of civilian population, particularly by increasing the sample size and including elders.

VI. Conclusions

1. The results of this thesis add to the growing body of literature that proves that, despite the widely held belief in the clinical environment, mTBI should not be considered a benign incident.

2. In this cohort of previously healthy working-age adults, with few known aggravating factors (no neurological or psychiatric history, no previous TBI, no record of alcohol or drugs abuse disorder), concussion induced an altered global cognitive state that was detectable on the day of the injury and that persisted up to at least 2 weeks. The global cognitive dysfunction was accompanied by multiple indicators of neuropsychological impairment, as observed in comparison with the control group of age-, sex-, and education-matched participants at 1-2 weeks following concussion.

3. Following concussion, normal CT scan findings should not be interpreted as a definite absence of structural brain injury. Brain lesions indicative of diffuse axonal injury were visible through magnetic resonance imagining in 10% of the patients. Other 25% of the cases presented abnormalities, not visible on the CT image, that could have functional repercussions, regardless of their uncertain traumatic etiology.

4. Our results support the use of SCAT2 as a suitable tool for performing a standardized examination of symptoms and signs of concussion, in the emergency departments and in civilian follow-up assessments. As routine examination should aim at incorporate factors with predictive value for identifying patients at risk of protracted recovery, tools like SCAT can prove valuable.

5. The presence of concussion-like symptoms in the general population is substantially elevated: only 10% of the participants in the control group did not endorse any symptom. Furthermore, 58.3% of them reported 3 or more concussion-like complaints, which is a frequent criterion for identifying PPCS after mTBI. Based on the established reference interval, the threshold that allows for a distinction between cases is 10 symptoms.

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VIII. Supplementary Material

A. English version of STUDY I.

B. Is it possible to screen for patients at high risk of developing postconcussive syndrome? Results of a pilot study using serum biomarkers and clinical variables

C. Longitudinal results of the neuropsychological examination

D. Genetic vulnerability for an incomplete recovery following mTBI

E. Conclusions (for the Supplementary Material)

F. References

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ORIGINAL ARTICLE

Neuropsychological alterations and neuroradiological findings in patients with post-traumatic concussion: results of a pilot study☆

A. Rădoi[a], M.A. Poca[a,b,*], V. Cañas[a], J.M. Cevallos[b], L. Membrado[c], M.C. Saavedra[c], M. Vidal[a], F. Martínez-Ricarte[a,b], J. Sahuquillo[a,b]

[a] *Unidad de Investigación de Neurotraumatología y Neurocirugía (UNINN), Institut de Recerca Vall d'Hebron (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain*
[b] *Servicio de Neurocirugía, Hospital Universitario Vall d'Hebron, Barcelona, Spain*
[c] *Urgencias de Neurotraumatología, Hospital Universitario Vall d'Hebron, Barcelona, Spain*

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KEYWORDS
Mild traumatic brain injury;
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Susceptibility-weighted imaging;
Post-concussion syndrome

Abstract
Introduction: Mild traumatic brain injury brain injury (mTBI) has traditionally been considered to cause no significant brain damage since symptoms spontaneously remit after a few days. However, this idea is facing increasing scrutiny. The purpose of this study is to demonstrate the presence of early cognitive alterations in a series of patients with mTBI and to link these findings to different markers of brain damage.
Methods: We conducted a prospective study of a consecutive series of patients with mTBI who were evaluated over a 12-month period. Forty-one (3.7%) of the 1144 included patients had experienced a concussion. Patients underwent a routine clinical evaluation and a brain computed tomography (CT) scan and were also administered a standardised test for post-concussion symptoms within the first 24 h of mTBI and also 1-2 weeks later. The second assessment also included a neuropsychological test battery. The results of these studies were compared to those of a control group of 28 healthy volunteers with similar characteristics. Twenty patients underwent a magnetic resonance imaging (MRI) scan.

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* Corresponding author.
E-mail address: pocama@neurotrauma.net (M.A. Poca).

Results: Verbal memory and learning were the cognitive functions most affected by mTBI. Seven out of the 20 patients with normal CT findings displayed structural alterations on MR images, which were compatible with diffuse axonal injury in two cases.
Conclusions: Results from this pilot study suggest that early cognitive alterations and structural brain lesions affect a considerable percentage of patients with post-concussion syndrome following mTBI.

PALABRAS CLAVE
Traumatismo craneoencefálico leve;
Déficits neuropsicológicos;
Lesión axonal difusa;
Sport Concussion Assessment Tool 2;
Susceptibility weighted imaging;
Síndrome posconmocional

Alteraciones neuropsicológicas y hallazgos neurorradiológicos en pacientes con conmoción cerebral postraumática. Resultados de un estudio piloto

Resumen
Introducción: Los traumatismos craneoencefálicos leves (TCE-L) han sido tradicionalmente considerados acontecimientos sin repercusiones cerebrales significativas, cuya sintomatología remite espontáneamente en unos días. Sin embargo, estos hechos son cada vez más cuestionados. Este estudio pretende objetivar la existencia de alteraciones cognitivas precoces en una serie de pacientes con TCE-L y relacionar los hallazgos con distintos marcadores de lesión cerebral.
Métodos: Estudio prospectivo de una cohorte de pacientes con un TCE-L valorados de forma consecutiva durante 12 meses. De un total de 1.144 pacientes, se seleccionó a 41 (3,7%) que habían presentado una conmoción cerebral. Además de la valoración clínica habitual y de la práctica de una tomografía computarizada (TC) cerebral, los pacientes fueron estudiados mediante un test estandarizado para síntomas posconmocionales en las primeras 24 h después del TCE-L y al cabo de 1-2 semanas y, coincidiendo con la segunda valoración, mediante una batería neuropsicológica. Los resultados se compararon con los de un grupo de 28 voluntarios sanos de características parecidas. En 20 pacientes se practicó una resonancia magnética (RM) craneal.
Resultados: En este análisis exploratorio, la memoria y el aprendizaje verbal fueron las funciones cognitivas más afectadas después del TCE-L. Siete de los 20 pacientes con TC cerebral normal presentaron alteraciones estructurales visibles por RM, que en dos casos fueron compatibles con la presencia de lesión axonal difusa.
Conclusiones: Los resultados de este estudio piloto sugieren la presencia de alteraciones cognitivas precoces y lesiones cerebrales estructurales en un porcentaje no despreciable de pacientes que han presentado una conmoción cerebral recuperada después de un TCE-L.

Introduction

Traumatic brain injury is highly prevalent in both industrialised and developing countries, with an estimated annual incidence of between 150 and 250 cases per 100 000 population.[1] In terms of severity, 10% of cases are severe (Glasgow Coma Scale [GCS] score $\leq$8), 10% are moderate (9-13), and 80% are mild (14 or 15).[2]

Little attention has historically been dedicated to the consequences of mild traumatic brain injury (mTBI), as it is considered an essentially reversible condition, with no detectable brain injury and few or no residual sequelae. However, recent years have seen the publication of numerous studies questioning this belief. In the hospital context, management protocols for mTBI typically establish that patients with normal CT findings may be discharged, often with no clinical follow-up. However, there is evidence that in as many as 25% of cases with normal CT findings, brain MRI does display alterations.[3]

In addition to GCS score (14-15), the traditional diagnostic criteria for mTBI are loss of consciousness (lasting <30 min) and post-traumatic amnesia (PTA; duration of less than 24 h). When any of these conditions is present, patients are considered to have concussion.[4] The consequences of mTBI are variable, ranging from the total absence of sequelae to an array of symptoms including headache, dizziness, nausea, gait instability, irritability, memory alterations, or difficulty concentrating. Approximately 30% of patients do not fully recover within 3 months of the trauma[5]; this is known as post-concussive syndrome.[6]

Despite advances in techniques for identifying brain injury, most studies acknowledge that some proportion of patients present persistent, incapacitating symptoms after MHT, despite normal neuroimaging findings. This explains why many authors believe that brain injury may not be the

Table 1 Criteria used to identify concussion in patients attended within the first 24 h after mTBI. Study inclusion required that patients display at least one of the following indicators, which were classified dichotomously (yes/no).

Indicator	Remarks
Loss of consciousness	Witness confirmation was required. Must be differentiated from syncopal episodes
PTA	Evaluation of the detailed account of events occurring immediately before and after the mTBI
Seizures	Objective post-concussive symptoms
Vomiting	
Severe post-concussive symptoms	A 0-4 scale was used to record severity of symptoms: headache, nausea, feeling of instability, hypersensitivity to light, hypersensitivity to sound, disorientation, blurred vision, and dizziness Symptoms were classified as severe if any scored $\geq$3 points or if the sum of all symptoms was $\geq$5

only cause of long-term alterations in some patients following mTBI. Residual sequelae in these patients may be influenced by a series of conditioning factors, such as personality traits, existing systemic or mental health disorders, comorbidities (chronic pain, anxiety or depressive disorders, etc.), sociopsychological factors, or the patient's involvement in legal claims.[7]

In the classic view, the great majority of neuropsychological alterations secondary to moderate or severe head trauma can be explained by the location of the associated brain lesions.[8] However, in cases where brain CT scans do not clearly show focal lesions, cognitive dysfunction may be explained by the disconnection of various brain structures due to diffuse axonal injury (DAI).[5] This phenomenon may also explain the presence of residual symptoms and cognitive alterations following mTBI. The most frequent neuropsychological sequelae of mTBI include alterations in information processing speed, attention, and memory.[9–12]

The susceptibility-weighted imaging (SWI) sequences included in modern MRI protocols aid the diagnosis of potential structural lesions in mTBI.[13] SWI is extremely sensitive to paramagnetic elements and is particularly useful for identifying microbleeds. Some studies have shown the technique to be up to six times more effective than T2*-weighted sequences in detecting punctiform microbleeds associated with DAI.[14]

This pilot study aims to evaluate the early presence (<14 days after trauma) of cognitive, affective, and behavioural symptoms in a series of patients with concussion secondary to mTBI and in a group of healthy controls and to explore the potential association between the cognitive deficits observed and the clinical symptoms. We also aimed to determine brain injury severity through MRI analysis of structural lesions in a subgroup of patients.

Patients and methods

Patient and control groups

The patients included in the study were treated between April 2013 and April 2014 at the neurotraumatology unit of Vall d'Hebron University Hospital's emergency department. To be included in the study, patients had to meet all the inclusion criteria and none of the exclusion criteria listed below:

- *Inclusion criteria*: (1) age between 18 and 65 years; (2) being a fluent speaker of Catalan or Spanish; (3) having had mTBI with a GCS score of 14-15 in the 24 h prior to study inclusion; (4) having experienced concussion, identified by loss of consciousness lasting <30 min (verified by a witness), vomiting, seizures, PTA lasting <24 h, or intense post-concussive symptoms (Table 1); (5) normal neurological examination findings; and (6) normal brain CT findings.
- *Exclusion criteria*: (1) previous head trauma requiring hospital care; (2) history of chronic substance abuse; (3) known psychiatric or neurological condition; (4) chronic systemic disease with potential cognitive effects (renal insufficiency or kidney failure, metabolic syndrome, etc.); and (5) polytrauma with an Injury Severity Scale score above 6.

Neurological examinations and initial analyses of brain CT scans were performed by the on-call neurosurgeon; these are routine procedures in the assessment of mTBI. Brain CT scans obtained in the emergency department were subsequently reassessed by neuroradiologists.

Participants of the control group were companions, and family members of patients admitted to the neurosurgery department. Of the candidates interested in participating, we selected those who met inclusion criteria 1 and 2 and none of the exclusion criteria. Volunteers were also matched to patients for age and level of education. The final control group comprised 28 volunteers (18 men and 10 women) with a median age of 29 (interquartile range [IQR], 21; range, 18-64).

All patients and controls signed informed consent forms approved by Vall d'Hebron University Hospital's Ethics Committee (PR-AG-47-2013).

Assessment and follow-up procedures

In addition to the initial clinical assessment, all patients were evaluated with CT scanning and a standardised test for post-concussion symptoms within 24 h of the trauma. Thirty-four patients were also evaluated a second time within 2 weeks of the trauma. Brain MRI scans were also performed for a subgroup of 20 patients (14 men and 6 women; median

Table 2 List of tests in the neuropsychological assessment battery.

Target	Test
Memory	Rey Auditory Verbal Learning Test[19]
	Brief Visual Memory Test—Revised[20]
Attention and information processing speed	Trail Making Test,[21] part A
	Conner's Continuous Performance Test II, v. 5.2[22]
	Wechsler Adult Intelligence Scale (WAIS-III),[23] digit symbol-coding subtest
	WAIS-III, symbol search subtest
Executive function	Controlled Oral Word Association Test[21]
	Trail Making Test, part B
	WAIS-III, working memory subtests (letter-number sequencing; digit span)
Effort	Test of Memory Malingering[17]

age, 29; IQR, 21; range, 18-64). All controls were assessed once only.

Standardised concussion assessment

Patients were assessed with the Sport Concussion Assessment Tool 2 (SCAT2)[15] within 24 h of the mTBI during their stay at the emergency department's neurotraumatology unit and subsequently during the neuropsychological examination. SCAT2 is a standardised assessment tool designed to measure the acute effects of concussion incurred during sport. The test records post-concussive symptoms, loss of consciousness (with confirmation from witnesses), and GCS score; it also involves an assessment of balance and coordination and a cognitive evaluation, performed using the Standardized Assessment of Concussion (SAC) tool. The SAC evaluates temporal orientation, immediate and delayed memory, and concentration. The SCAT2 is particularly useful in clinical contexts as it is quick to administer and addresses multiple dimensions.[16] The maximum scores for the SAC and the SCAT2 are 30 and 100, respectively.

Neuropsychological assessment

All participants underwent neuropsychological assessments on one occasion; patients were evaluated within the first 2 weeks after the mTBI. Cognitive function was assessed using a neuropsychological battery testing attention, memory, information processing speed, and complex executive functions (Table 2). Tests were selected in line with the recommendations of the National Institute of Neurological Disorders and Stroke.[2] The tests selected are included in the Institute's Core Data Elements for studying patients with traumatic brain injury.[2] Effort was tested using the Test of Memory Malingering (TOMM)[17] in order to detect any feigned symptoms or a lack of collaboration during the examination. Patients also completed the Hospital Anxiety and Depression Scale,[18] as anxiety and depression can influence cognitive profiles. The duration of the complete cognitive examination was approximately 120 min, including a 5-10-min rest period, where needed. These studies were performed by researchers with specific training in neuropsychology (A.R. and V.C.).

Magnetic resonance imaging evaluation

The MRI study was performed using a SIEMENS Magnetom Trio Tim syngo 3T MRI scanner at Hospital Clínic de Barcelona's Centre for Diagnostic Imaging. Images were analysed by a neuroradiology expert (N.B.) who was not a member of the study team. For each patient, we obtained a high-resolution, T1-weighted structural image (3D magnetisation-prepared rapid gradient echo [MP-RAGE]) and T2-weighted FLAIR and gradient echo sequences. Microbleeds were identified using SWI sequences. Sequences were obtained in the same order for all participants. These studies were performed within 14 days of the mTBI.

Statistical analysis

All variables were analysed using version 22 of the SPSS statistics package (Chicago, Illinois, USA). As most variables did not follow a normal distribution, non-parametric tests (the χ^2-test and the Mann—Whitney *U*-test) were used to compare and analyse associations between variables.

In the neuropsychological assessment, in which analysis involves many related variables, the likelihood of type I error is known to increase when multiple statistical comparisons are made. We may therefore consider applying the Benjamini—Hochberg correction[24] for the false discovery rate, or using a more conservative significance threshold ($P < .01$). However, the small sample size causes a considerable reduction in statistical power. We therefore decided on a more liberal statistical approach, assuming a 5% likelihood of error for all results. In order to better describe the magnitude of the differences identified, effect size was calculated with the correlation coefficient *r*.

Results

The neurosurgery department treated 1144 patients diagnosed with mTBI during the study period. The majority of these patients were elderly or had incurred minor head trauma not associated with loss of consciousness, PTA, or other relevant symptoms. We selected a total of 41 patients (16 women and 25 men; median age, 34; IQR, 24; range, 18-64) (Fig. 1) according to the protocol described above. For all patients, we performed a brief cognitive assessment and recorded post-concussive symptoms (with the SCAT2) the day the trauma occurred; results were compared with those of the control group. As 7 patients (17%) did not attend the follow-up visit, the extensive follow-up cognitive assessment was only performed for the remaining 34 patients (12 women and 22 men; median age, 32.5; IQR, 23; range, 18-64).

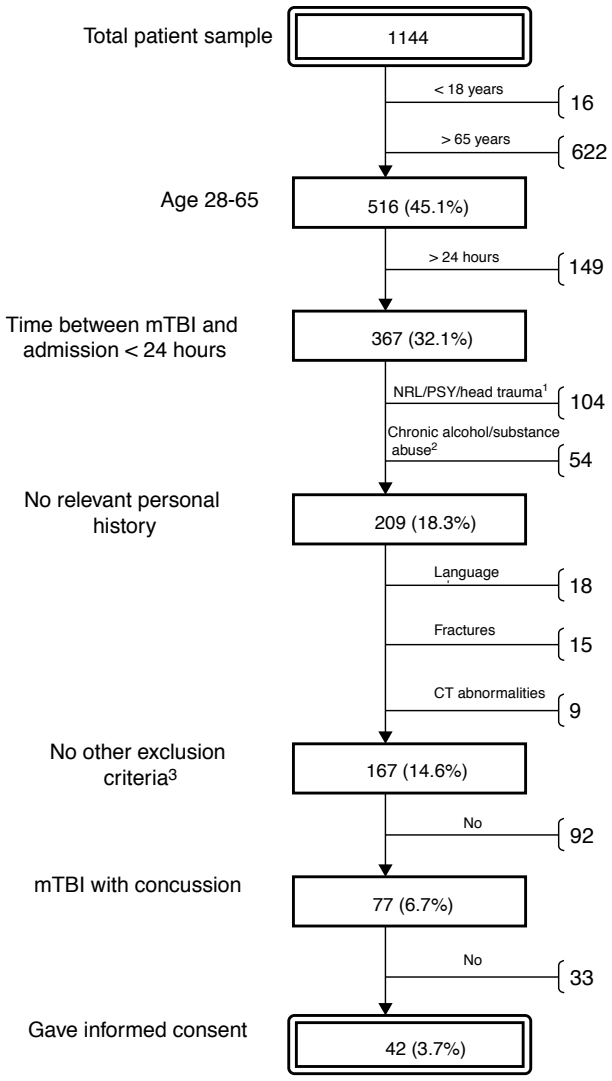

Figure 1 Algorithm for the selection of patients with mTBI treated during the study period at the Vall d'Hebron University Hospital emergency department's neurotraumatology unit. Percentages refer to the total number of patients. Reasons for exclusion: previous neurological/psychiatric condition or traumatic brain injury (1); chronic alcohol or substance abuse (2); other (3): insufficient level of Spanish or Catalan, fractures requiring admission to hospital, and pathological brain CT findings.

Application of the Sport Concussion Assessment Tool 2 and the Hospital Anxiety and Depression Scale

As expected, patients displayed significantly more symptoms and significantly greater symptom severity than controls in the hours following the mTBI ($z = -4.44$, $P < .001$, $r = 0.53$, vs $z = -4.88$, $P < .001$, $r = 0.58$, respectively) (Table 3). Significant intergroup differences were also observed for overall SCAT2 score ($z = 3.46$, $P < .001$, $r = 0.43$).

In the clinical follow-up examination performed several days after the trauma, there continued to be a significant difference between groups for number and severity of

Table 3 Longitudinal evaluation of SCAT2 scores in patients and controls.

	mTBI		Controls	As.	As.	As.
	As. 1[a] ($n = 41$)	As. 2[b] ($n = 34$)	($n = 28$)	1—As. 2	1—controls	2—controls
	Median (IQR, range)	Median (IQR, range)	Median (IQR, range)	z (*P*)	z (*P*)	z (*P*)
Number of symptoms	8 (6, 0-17)	8 (11, 0-20)	3 (4, 0-11)	0.13 (0.89)	0.13 (<0.001)***	3.45 (<0.001)***
Severity	19 (28, 0-71)	13 (36, 0-68)	5 (9, 0-31)	1.07 (0.28)	4.88 (<0.001)***	3.22 (0.001)**
SAC	27 (4, 22-30)	25 (4, 17-29)	27 (4, 22-30)	0.04 (0.96)	2.98 (0.003)**	2.77 (0.006)**
SCAT2[c]	80 (9, 45-94)	81 (15, 55-93)	87.5 (11, 75-96)	0.66 (0.51)	3.46 (<0.001)***	2.80 (0.005)**

[a] As. 1 refers to the acute-phase assessments performed within 24 h of mTBI.
[b] As. 2 refers to the second assessment performed a median of 5 days (range, 2-13) after trauma.
[c] SCAT2 score could not be calculated for patients who were unable to perform the balance assessments; this explains the difference in the number of patients assessed in the baseline examination ($n = 35$) and the follow-up examination ($n = 31$).
As.: assessment; IQR: interquartile range; mTBI: mild traumatic brain injury;fAs expected, patients displayed significant SAC: Standardized Assessment of Concussion; SCAT2: Sport Concussion Assessment Tool 2.
** $P < .01$.
*** $P < .001$.

Table 4 Relevant sociodemographic and clinical variables of the patient and control groups.

	mTBI ($n = 34$)	Controls ($n = 28$)	z (P)
Sex (M/F)	22/12	18/10	0.001 (.97)[a]
Age (years)	34 (24, 18-64)	29 (21, 18-64)	−0.58 (.56)
Years of schooling	14 (6-22)	13.5 (8-22)	−0.32 (.74)
Laterality: right/left/ambidextrous	30/3/1	26/0/2	–
Loss of consciousness	22 (64.70%)		
PTA	26 (76.47%)		
GCS score (15/14)	33/1		

Median, interquartile range, and range are shown in brackets.
GCS: Glasgow Coma Scale; mTBI: mild traumatic brain injury; PTA: post-traumatic amnesia.
[a] The chi square test was used to compare the sex distribution between groups.

symptoms and overall SCAT2 score ($z = -3.45$, $P < .001$, $r = 0.44$; $z = 3.22$, $P < .001$, $r = 0.41$; and $z = 2.80$, $P = .005$, $r = 0.36$, respectively). However, Hospital Anxiety and Depression Scale scores showed no significant difference between groups for the anxiety or depression subscales ($z = -0.59$, $P = .55$; and $z = -0.68$, $P = .50$, respectively).

Neuropsychological assessment

The 34 patients who attended the follow-up visit underwent an extensive neuropsychological assessment; results were compared to those of the control group. Most patients were assessed within the first week after the mTBI (median, 5 days; range, 2-13) (Table 4). Table 5 displays the most relevant results from the cognitive assessment of the groups studied. As is shown in the table, sample size changed between tests, as patients with mild injuries to their dominant arm did not perform tests requiring manual skills, such as drawing or psychomotor speed tasks.

The Rey Auditory Verbal Learning Test showed the most marked difference between groups. Patients' performance was significantly poorer than that of controls in learning and immediate and delayed verbal memory, with a medium effect size ($r > 0.3$). Scores for visual learning and delayed visual memory were also lower in patients than in controls, although intergroup differences in delayed visual memory scores displayed only a trend toward statistical significance ($P = .054$). Patients also scored significantly lower than controls for 3 indicators (working memory span, digit span subtest, and number of perseverations in the Continuous Performance Test). These variables shed light on important aspects of attention and executive function (due to working memory and inhibition components) that appear to be affected several weeks after mTBI.

It should be noted that given the high number of variables analysed, any more conservative statistical strategy involving correction for multiple comparisons would require a significance threshold of approximately $P < .003$. The alterations observed in our cohort had a significance level of .005 or higher.

Magnetic resonance imaging findings

Brain MRI studies were performed for a subgroup of 20 patients, selected on the basis of their availability for testing. MRI was performed a median of 6 days after trauma occurred (range, 1-13). Table 6 provides a detailed description of demographic characteristics and neuroradiology findings for this subgroup.

Although all patients showed normal brain CT findings at baseline, MRI revealed lesions suggestive of DAI in 2 patients (10%). Figure 2 shows the lesions observed in a 26-year-old patient with an mTBI with loss of consciousness and PTA following a motorcycle accident. We also observed focal signal alterations of potentially traumatic aetiology in five other patients (25%); aetiology could not be confirmed in all cases, however.

Discussion

The results of this pilot study demonstrate that concussive symptoms secondary to mTBI may last days after the trauma occurs potentially interfering with patients' work or academic performance. Besides the typical symptoms of concussion (headache, vertigo, etc.), these patients may display cognitive alterations, which are observable through the use of specific tools. Finally, MRI revealed structural alterations compatible with DAI or microbleeds in a considerable percentage of patients, despite normal early brain CT results in all patients.

Remarks on the inclusion of patients in the study

One of the most noteworthy findings was the high number of patients attending the hospital following MHT who were eventually found not to be eligible for inclusion. Age was the most frequent reason for exclusion: 55% of patients treated during the study period were older than 65. This figure is consistent with the recent changes observed in the epidemiology of traumatic brain injury. A significant increase has been observed in the ages of trauma patients worldwide, with falls surpassing traffic accidents as the most frequent cause in this age group.[1] The strict screening criteria applied, specifically chosen to eliminate the known confounding factors, reduced the number of potential participants to 6.9% of the patients treated. Only 60% of those meeting the inclusion criteria volunteered to participate in the study. Therefore, the study participants accounted for less than 4% of all patients treated in a 1-year period at this tertiary hospital. In a 2013 study, Luoto et al.[25] discuss this issue and note that many hospital studies into mTBI use

Table 5 Comparative analysis of neuropsychological test results for patients and controls.

	MHT		Controls ($n = 28$)		
	n	Median (IQR, range)	Median (IQR, range)	z (P)	r
TMT A	32	32.5 (19, 19-86)	30 (11, 13-54)	−1.632 (.103)	0.21
TMT B	29	65 (47, 34-143)	61 (20, 24-150)	−0.559 (.576)	0.07
TMT proportional score[a]	29	1 (0.82, 0.36-2.59)	1.03 (0.93, 0.32-3.5)	−0.439 (.661)	0.06
Verbal learning	34	49.5 (10, 27-68)	57 (12, 39-71)	−2.812 (.005)**	0.36
Immediate verbal memory	34	11.5 (5, 6-15)	13.5 (3, 6-15)	−2.596 (.009)**	0.33
Delayed verbal memory	34	11 (4, 5-15)	13.5 (4, 8-15)	−2.802 (.005)**	0.36
Digit symbol-coding subtest	31	73 (25, 29-113)	83 (29, 48-120)	−1.693 (.090)	0.22
Symbol search	32	38.5 (11, 15-54)	39.5 (14, 25-51)	−0.519 (.604)	0.07
Attention span	34	6 (1, 4-9)	6 (2, 4-9)	−1.282 (.200)	0.16
Working memory span	34	4 (2, 2-7)	5 (2, 3-8)	−2.366 (.018)*	0.30
Digit span	34	14 (6, 7-23)	16 (4, 9-26)	−2.202 (.028)*	0.27
Letter-number sequencing	31	11 (4, 5-17)	11.5 (3, 7-16)	−0.972 (.331)	0.12
Immediate visual memory	32	6 (3, 1-11)	7 (6, 0-11)	−1.837 (.066)	0.24
Visual learning	32	25 (9, 7-33)	29.5 (9, 7-35)	−2.483 (.013)*	0.32
Delayed visual memory	32	10 (4, 3-12)	11.5 (2, 4-12)	−1.925 (.054)	0.25
Semantic fluency	34	24 (8, 16-40)	24 (9, 13-37)	−0.312 (.755)	0.04
Phonetic fluency	34	43 (14, 23-59)	46 (21, 20-80)	−1.196 (.232)	0.15
Omissions (CPT)	34	1 (3, 0-30)	1 (3, 0-13)	−0.007 (.994)	<0.01
Commissions (CPT)	34	13 (12, 2-31)	11 (10, 1-26)	−1.711 (.087)	0.22
Reaction time (CPT)	34	402 (87, 306-583)	391 (48, 332-588)	−0.087 (.931)	0.01
Perseverations (CPT)	34	0 (2, 0-23)	0 (0, 0-4)	−2.292 (.022)*	0.29

CPT: Continuous Performance Test; MHT: mild head trauma; TMT: Trail Making Test.
[a] TMT proportional score = (TMT B − TMT A)/TMT A.
* $P < .05$.
** $P < .01$.
r = effect size (0.5: large; 0.3: medium; 0.1: small).

potentially biased samples, for which reason results cannot be generalised to the entire population of trauma patients.

Diagnostic criteria for mTBI

According to the classic 2004 World Health Organization criteria,[4] diagnosis of mTBI requires a GCS score of 13, 14, or 15. However, more recent diagnostic criteria rule out patients scoring 13 on the GCS. Various authors note that in terms of mortality indicators and complications, the progression of patients scoring 13 is more similar to that of patients with moderate than with mTBI.[26] Stein and Ross[27] compare initial brain CT findings in a group of 106 patients scoring 13 on the GCS to those of 341 patients diagnosed with moderate traumatic brain injury according to the classic criteria (GCS score 9-12). Both groups showed a similar prevalence of lesions in the brain CT images (44.3 and 40.3%, respectively). These researchers also found that 20% of patients with pathological brain CT findings required surgical treatment; for this reason, they propose that trauma patients scoring 13 on the GCS should be reclassified as having moderate rather than mTBI.[27]

The present study also required normal brain CT findings among the inclusion criteria applied. The exclusion of patients with pathological brain CT findings ensures a more homogeneous sample, as patients displaying brain injury on CT scans are followed up according to different protocols, which are more similar to those used for cases of moderate traumatic brain injury. The absence of brain injury in conventional emergency department examination tests enabled us to study patients with more benign symptoms (GCS scores of 14 or 15 and normal CT results).

Clinical assessment of mTBI: current tools

In order to address the issue of why some patients present post-concussive syndrome, it is necessary to better understand the clinical presentation of acute-phase mTBI. The prognostic value of such traditional descriptors as presence and duration of loss of consciousness and PTA are not well established; neither phenomenon is well understood from a pathophysiological perspective in the context of mTBI. The validity and predictive power of PTA is very uncertain in these patients, despite being a defining characteristic of concussion and a critical element of routine hospital assessment.[16]

We used the SCAT2, originally designed to assess trauma in sport, to obtain a complete register of participants' clinical information. Patients continued to present a high number of post-concussive symptoms at 1-2 weeks after trauma, although severity tended to decrease. The SCAT2 results at the time of hospitalisation and at 1-2 weeks support the use of the tool in the standardised monitoring of post-concussive symptoms, as it also provides a general overview of these patients' cognitive status. However, evaluation of the clinical symptoms assessed in this study (loss of consciousness

Table 6 Demographic, clinical, and neuroradiological characteristics of the 20 patients who underwent MRI studies, ordered by SAC score.

No.	Age/sex	Cause of head trauma	Initial evaluation (<24 h)			MRI findings
			No. symptoms	SAC	SCAT2	
1	64/F	Fall	15	20	67	Subcortical WM involvement (Fazekas 2) Small choroidal fissure cyst
2	26/F	Traffic accident (motorcyclist)	10	20	49	Small left-hemisphere subcortical WM lesions, some with microbleeds, indicative of DAI
3	43/M	3-m fall	8	20	82	Small isolated WM lesions of little pathological importance, with no microbleeds
4	52/M	Workplace accident	15	20	62	Some small, non-specific lesions to the frontal subcortical WM
5	20/M	Sports accident	10	22	79	No remarkable findings
6	50/M	Traffic accident (cyclist)	2	23	86	Bilateral, predominantly frontal lesions, suggestive of DAI, with associated microbleeds
7	42/F	Traffic accident (motorcyclist)	0	25	74	Small microbleed on the left side of the pons. Signal alteration in the periventricular WM (Fazekas 1)
8	30/F	Fall	12	25	80	No remarkable findings
9	28/M	Assault	13	25	—[a]	No remarkable findings
10	56/F	2-m fall	2	25	82	No remarkable findings
11	38/M	Assault	9	26	77	Single posterior temporal focal microbleed
12	27/M	Traffic accident (collision)	8	26	82	No remarkable findings
13	22/M	Assault	1	26	74	No remarkable findings
14	27/F	Traffic accident (motorcyclist)	8	27	88	No remarkable findings
15	24/M	Sports accident	7	27	84	No remarkable findings
16	46/M	Traffic accident (motorcyclist)	6	27	86	Small, non-specific lesions to the subcortical and periventricular WM, with no focal microbleeds
17	18/M	Sports accident	9	27	84	No remarkable findings
18	19/M	Sports accident	7	28	83	No remarkable findings
19	30/M	Sports accident	7	29	—[a]	No remarkable findings
20	24/M	Sports accident	4	30	94	No remarkable findings

DAI: diffuse axonal injury; MRI: magnetic resonance imaging; SAC: Standardized Assessment of Concussion; SCAT2: Sport Concussion Assessment Tool 2; WM: white matter.

[a] It was not possible to test balance in patients who were unable to leave bed, for which reason SCAT2 scores could not be calculated.

and balance) is less reliable in the hospital context civilian mTBI than in sport, as it is often based on information provided by patients themselves or on witness accounts. Furthermore, balance cannot be assessed in patients with injuries to the lower limbs which prevent them from standing. Results of balance assessments are more variable in the general adult population than among young athletes: 10% of a sample from a healthy Canadian population and approximately 65% of a Finnish control group had low scores for balance tests.[16] These results suggest that we should reconsider the value of the SCAT2 balance subtest in future studies aiming to validate the scale's use outside the context of sport.

Neuropsychological evaluation results

While traumatic brain injury can affect almost any aspect of brain function, the most significant consequence is probably dysfunction of frontal systems, which are of particular importance for executive function (planning and organisation, working memory, flexibility, and behavioural inhibition, among others). For this reason, researchers such as Chen and d'Esposito[28] and Stuss[29] define traumatic brain injury as a disorder of cognitive control.

The results of our detailed neuropsychological evaluation of patients with mTBI and controls show that learning SIN capacity and memory are mildly impaired in the first 2 weeks after an mTBI. Other indicators of attention (which also have an executive component, due to the involvement of working memory and inhibition) add to the results that show the presence of subtle (...) subtle cognitive deficits in this group of patients.

These are the results of an exploratory analysis, and statistical significance for differences between the patient and control groups was set at $P < .05$, with no strict post hoc correction. However, they are consistent with the results of a recent systematic review, which concluded that cognitive

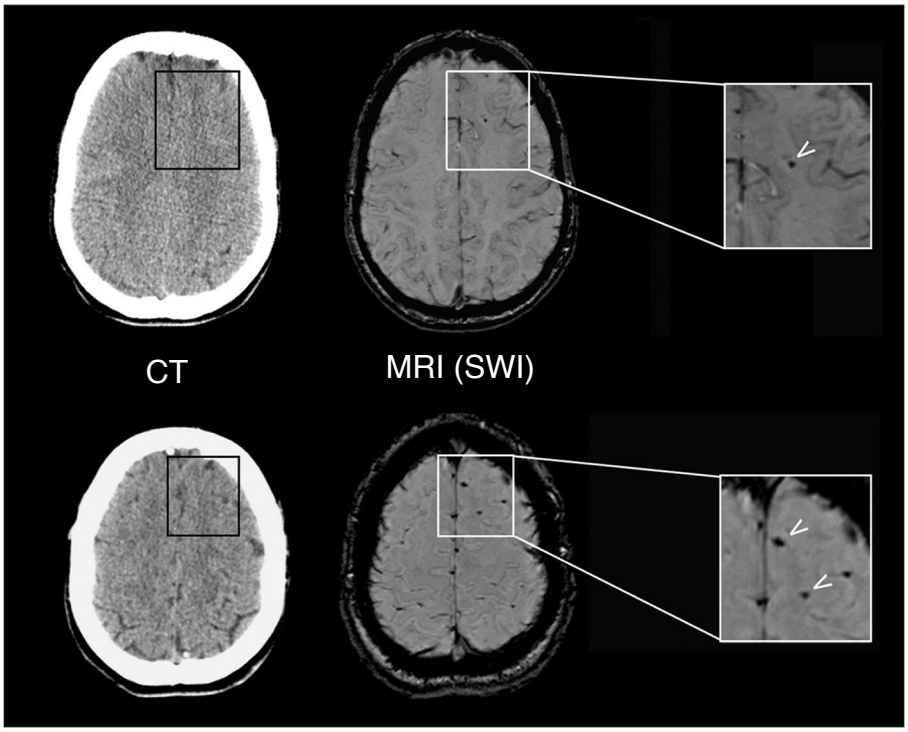

Figure 2 Neuroradiological findings in a 26-year-old patient with mTBI. The results of a brain CT scan (left) performed 2 h after the trauma were normal, whereas a brain MRI study at 10 days (right) revealed focal signal alterations (arrows) on SWI sequences, which corresponded to microbleeds indicative of mild DAI.

deficits are consistently associated with mTBI in the first 2 weeks after trauma occurs.[11] The study also found a limited association between loss of consciousness and reduced information processing speed. However, while nearly 70% of the patients in our study had experienced loss of consciousness, the results do not clearly confirm impairment of information processing speed.

Normal brain computed tomography findings and lesions on the magnetic resonance images

Patients with mTBI, and particularly those with persistent symptoms, may present brain injuries that can go unnoticed on brain CT scans. SWI sequences identified traumatic structural lesions in 10% of the patients who underwent MRI studies. In another five patients (25%), MRI displayed lesions which were not observable on conventional brain CT scans, although aetiology was unclear in some cases.

Although other studies have found a correlation between the total volume of lesions detected on SWI sequences and clinical indicators of severity,[30] no clear association has been established between the presence of these lesions and cognitive recovery following trauma. Several studies report that the presence of lesions in neuroimaging studies of patients with mTBI is associated with poorer results for such cognitive functions as memory. However, as the great majority of the neuropsychological tests applied yield similar results for groups of patients with and without lesions observable through neuroradiology, some authors assert that these patients do not require different treatment.[31]

These brain lesions occur during the acute phase of mTBI and persist indefinitely. Identification of these lesions provides information not only about severity, but also about which neurobehavioural systems may be affected. Advanced MRI studies provide information on the distribution of a brain injury and enable the creation of more effective assessment and treatment strategies, similar to the role these studies play in the rehabilitation of patients with ischaemic stroke.

In conclusion, despite our study's limitations, and contrary to the received wisdom in the clinical setting, our results confirm that some cases of mTBI should not be considered banal injuries. Despite normal CT findings, advanced MRI studies showed that 10%-35% of patients had lesions that were potentially indicative of DAI. Both during the acute phase and at 1-2 weeks, our patients displayed alterations in their overall neurocognitive status, compared to the control group. The results of the neuropsychological evaluation show that these patients' cognitive status continues to be affected in the medium term, with symptoms including memory and executive attention issues. One of the study's main limitations is its relatively short follow-up period. As symptoms can persist for months following mTBI, potentially even becoming permanent, longer follow-up periods are necessary. Future studies should address this matter.

Our findings show that the typical management of patients attending hospital with mTBI (usually an assessment and discharge without follow-up) may not be appropriate in all cases. Structured recording of post-concussive symptoms and neuropsychological assessment provide very important information on the alterations these patients may display

for at least the first 2 weeks after trauma. Despite the need for larger samples, our results support the use of the SCAT2 questionnaire as part of routine clinical care.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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B. Is it possible to screen for patients at high risk of developing postconcussive syndrome? Results of a pilot study using serum biomarkers and clinical variables

Abbreviations

ACRM: American Congress of Rehabilitation Medicine; **AUC:** area under the curve; **BM**: biomarker; **BMI:** body mass index; **Casp-1**: caspase 1; **CI:** confidence interval; **CRP**: C-reactive protein; **CT**: computed tomography; **DSM-V:** Diagnostic and Statistical Manual of Mental Disorders, 5th edition; **DTI**: diffusion tensor imaging; **ED**: emergency department; **ELISA**: enzyme-linked immunosorbent assay; **FLAIR:** Fluid Attenuated Inversion Recovery; **GCS**: Glasgow Coma Scale; **IL-1 β**: interleukin 1 beta; **Lep**: leptin; **LOC**: loss of consciousness; **MP-RAGE:** 3D Magnetization Prepared Rapid Gradient Echo; **MRI:** magnetic resonance imaging; **mTBI**: mild traumatic brain injury; **MLR:** multiple logistic regression; **NSE:** gamma-specific enolase; **PTA**: posttraumatic amnesia; **PPC:** post-concussion syndrome; **PPCS:** persistent post-concussion symptoms; **RI:** reference interval; **SCAT2:** Sport Concussion Assessment Tool, 2nd edition; **TBI**: traumatic brain injury; **VEGF-A**: vascular endothelial growth factor A; **vWF**: von Willebrand factor.

INTRODUCTION

Concussion is a diagnostic entity that defines a mechanically-induced brain dysfunction at the mild end of the severity spectrum and is often considered equivalent to mild traumatic brain injury (mTBI). Concussion is a frequent consequence of traffic accidents, assault, sports or military deployment and has been acknowledged as a topic of intense public concern in the last fifteen years. [1,2] mTBI may translate into somatic symptoms —imbalance, headache, nausea— and may affect cognitive and emotional functioning, that in most cases resolve in a matter of days or few weeks.[3] However, some patients experience persistent symptoms in what has been referred as the post-concussion syndrome (PCS).

In most clinical settings, the diagnosis of PCS in adults is considered when the symptoms persist after three months. In the last revision of the International Classification of Diseases, ICD-10, PCS is defined as including several "…disparate symptoms such as headache, dizziness, fatigue, irritability, difficulty in concentration and performing mental tasks, impairment of memory, insomnia, and reduced tolerance to stress, emotional excitement, or alcohol".[4] However, in the most recent edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), this diagnosis has been replaced by the term "neurocognitive disorder due to traumatic brain injury".[5] The lack of agreement between diagnostic criteria has been remarked by many.[6-8] The DSM-V criteria have introduced additional confusion, as diagnosis is only applicable to patients that present loss of consciousness (LOC), posttraumatic amnesia (PTA), disorientation, or focal neurological signs.[5] However, the number of clinically relevant symptoms and their severity to consider the diagnosis of PCS is still an ongoing debate.[9] Some experts consider that even one persistent symptom warrants clinical attention, while others require at least three symptoms or a combination of symptoms from different clusters (somatic, cognitive, emotional, fatigue). In a previous article, we have shown that concussion-like symptoms may be frequently endorsed by the general population with no history of TBI.[10]

Research in blood-based TBI biomarkers (BMs) has rocketed over the last two decades and, as remarked by Papa and Wang, it continues to grow.[11]TBI entails functional and/or structural changes of the glial cells and neurons, hence brain injury BMs have been specifically designed to tag these cellular populations and show objective cell injury. S100β, a 21 kDa molecular-weight calcium-binding protein, is one of the most studied BMs for glial cell damage, while gamma-specific enolase (previously called neuron-specific enolase, NSE) is for neurons.[12-14] S100β is the only BM that has been considered reliable enough to modify clinical policies in mTBI. The Scandinavian guidelines for the management of mTBI introduced a restriction for computerized tomography (CT) scanning in the absence of elevated S100β serum levels.[15] The rationale for these guidelines was based on numerous studies that have shown that S100B is highly sensitive in the first hours after TBI for detecting patients with lesions in the CT scan.[16,17] NSE serum levels have also shown a good association with unfavorable outcome and complications following TBI, but, in multivariate models that included also S100ß levels, the additional predictive capabilities and clinical usefulness of the NSE have been questioned.[18] To date, the primary focus of TBI research has been on neurons with little emphasis on glial cells or other components of the neurovascular unit.[19] However, in the last decade it has been shown

that the whole neurovascular unit is an important target both for primary and secondary injuries in TBI and therefore BMs for endothelial damage should be explored and incorporated in diagnostic panels focused in defining the severity of brain damage.

Among the BMs of endothelial cell damage, the vascular endothelial growth factor (VEGF) is considered one of most powerful factors involved in angiogenesis and modulation of endothelial permeability, dilation and proliferation.[20] VEGF also enhances neurogenesis and angiogenesis and reduces lesion volume in rodent experimental models of TBI.[21] The von Willebrand Factor (vWF) is an endothelium-specific glycoprotein primarily involved in hemostasis in response to endothelial damage. Elevated vWF serum level is considered a marker of increased vascular permeability and it has been associated with unfavourable outcome in patients with severe TBI.[22,23]

The role of neuroinflammation after severe TBI is well established, however its relevance in the evaluation of mTBI has been traditionally neglected.[24] Experimental evidence has shown in animal models, that mTBI could activate systemic inflammatory process.[25] However, to the best of our knowledge only a few clinical studies have focused on the association between systemic inflammation and unfavourable outcomes following mTBI.[26] Moderate and severe TBI are accompanied by a vigorous expression of various pro- and anti-inflammatory molecules, such as TNF-α, IL-1β, IL-6, IL-8 and IL-10 that have been considered as prognostic BMs.[27] IL-1β is the most studied pro-inflammatory cytokine in response to brain trauma, it regulates the release of other cytokines but it is notoriously difficult to detect in humans.[28] Since the 2000s it is widely accepted that the adipose tissue is not only a long-term energy storage but secretes numerous proteins involved in the inflammatory response (adipokines).[29] Leptin (Lep) is an adipokine involved in the regulation of the feeding behavior through the brain, but it is also a pro-inflammatory adipokine that promotes the production of IL-6 and TNF-α by monocytes.[29] Furthermore, Lep is expressed in the pituitary and other specific areas of the brain in rat, pig, sheep and human.[30] Leptin levels increase in serum and adipose tissue in response to pro-inflammatory stimuli[29] and leptin enhances the production of C-reactive protein, as well as TNFα and IL-6. In 2010, Dong et al. reported that leptin was significantly elevated in patients with acute basal ganglia hematoma and that plasma levels greater than 34.1 ng/mL predicted 1-week mortality and poor functional outcome better than the hematoma volume.[31] In a cohort of 142 isolated pediatric severe TBI patients, elevated acute plasma leptin

levels were associated with mortality and unfavorable neurological outcome; the leptin's predictive value was similar to that of the Glasgow Coma Scale score.[32]

C-reactive protein (CRP) is another BM included in this study. CRP is an acute-phase protein synthesized by the liver and has been described as a highly sensitive but non-specific BM that increases following infection, trauma and surgery, among others.[33] In severe TBI, Hergenroeder et al. showed that serum CRP was overexpressed in severe TBI patients compared with age-matched controls.[34] In mTBI, Su et al. found that elevated baseline CRP levels were independently associated with the increased risk of PCS, psychological problems and cognitive impairment.[26]

Almost all civilian mTBI may return to the normal life few weeks after injury. However, a small minority of mTBI patients may experience persistent post-concussive symptoms (PPCS) months or even years after the traumatic event.[35] PPCS, including cognitive impairment, affect not only the young population but are also increasingly common in the elderly. The primary aim of this study was to establish whether a single determination of a set of predefined serum BMs, determined early after injury, together with clinical symptoms assessed in a systematic manner could be helpful in screening patients at high risk of PPCS at 3 months post-injury. We used a BM panel that examined endothelial dysfunction (VEGF and vWF) and the inflammatory response (IL1ß, leptin and CRP), together with a more conventional BM of glial cells (S100ß).[28,36] We used two different criteria for the definition of PCS and multiple logistic regression to screen patients with mTBI and potentially identify those at high risk of PCS. If clinicians could use in the emergency department evaluation a tool with high positive predictive value of patients at risk of developing PCS, they would be able to refer them to specific rehabilitation programs and determine distinct follow-up strategies for patients that will recover completely in a few weeks and those who will probably present long-term mTBI-related problems.

PARTICIPANTS AND METHODS

Setting and mTBI group

This was a prospective single-center observational study conducted between April 2013 and March 2017 in all patients with TBI—regardless of their severity— admitted to the Emergency

Department (ED) of the Traumatology Hospital at the Vall d'Hebron University Hospital. Our institution is a tertiary referral centre with a translational research program in TBI. Patients were considered eligible for recruitment if they fulfilled all the inclusion criteria and none of the following exclusion criteria. Inclusion criteria: 1) between 18 and 65 years of age; 2) proficient speaker of Spanish and/or Catalan; 3) GCS of 14 or 15; 4) mTBI presenting with concussion defined as at least one of the following criteria: witnessed loss of consciousness (LOC) less than 30 min, PTA less than 24 h, vomiting, seizures or any intense postconcussional symptoms, normal neurological examination and a normal CT scan. Exclusion criteria: 1) previous TBI; 2) history of chronic abuse of psychoactive substances or alcohol; 3) known psychiatric or neurologic disorder; 4) chronic systemic disease with known repercussions on the cognitive status by itself or its treatment (cancer, kidney or liver failure, metabolic syndrome, etc.) and 5) associated polytraumatism, defined as an Injury Severity Score > 6.

Control group

Next-of-kin or companions of patients admitted to the Neurosurgery Department were invited to take part into a study to form a control group. Potential candidates should be between 18 and 65 years old and proficient speakers of Spanish and/or Catalan. The following exclusion criteria were applied: 1) previous TBI; 2) history of chronic abuse of psychoactive substances or alcohol; 3) known psychiatric or neurologic disorder; 4) chronic systemic disease with known repercussions on the cognitive status by itself or its treatment (cancer, kidney or liver failure, metabolic syndrome, etc.). The recruitment process has been described in detail elsewhere.[37] Controls were also evaluated with the second edition of the Sport Concussion Assessment Tool (SCAT2) used to assess patients.[38] The study complied with the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Vall d'Hebron University Hospital (Protocol number: PR-AG-47-2013). The participants accepted all the procedures and signed informed consent forms.

Assessment procedures and follow-up

All patients were evaluated in the ED room with a standardized protocol for concussion within 24 h since injury. A blood extraction for BMs was performed at the time of the first evaluation. The standardized assessment of concussion was repeated at two follow-up visits scheduled within the first two weeks and three months after injury, as part of a broader outcome assessment that included functional and neuropsychological data. The protocol for the control

group included only one blood sampling extraction, the same concussion assessment described in mTBI patients and a magnetic resonance imaging (MRI) of the brain. MRI scanning was performed with a SIEMENS Magnetom® TrioTim syngo 3-tesla equipment (Siemens Healthineers AG, Munich, Germany); data from a high-resolution 3D Magnetization Prepared Rapid Gradient Echo (MP-RAGE) protocol in addition to Fluid Attenuated Inversion Recovery (FLAIR) and echo gradient T2 sequences were assessed and informed by an expert neuroradiologist. MRI results for the control participants have been described elsewhere.[37] In addition, the body mass index (BMI) was used to classify the cohort into underweight (<18.5 kg/m^2), normal-weight (between 18.5 and 24.9 kg/m^2), overweight (between 25 and 29.9 kg/m^2) or obese (>30 kg/m^2) categories.

Standardized assessment of concussion

Within one hour after recruitment, following routine neurological examination, patients were assessed using SCAT2.[38]. Although this tool was initially designed for sideline examination in sports-related concussion, it has been increasingly used in the clinical setting. In brief, the patient rates the presence/absence of 22 postconcussional predefined symptoms and evaluates their individual intensity on a Likert scale from 0 to 6, resulting in two scores: the number of endorsed symptoms at evaluation and their severity. The symptoms rated 1 or 2 in severity are considered mild, 3 or 4 moderate and 5 or 6 severe. The total severity score can range between a minimum of 0 to a maximum of 132. Items tapping orientation, working memory and verbal memory are summed up in a cognitive index known as Standardized Assessment of Concussion (SAC) score, with a minimum value of 0 and a maximum of 30 points. In all SCAT2 indices, a high score is indicative of a good performance. In addition to the number of symptoms and the SAC, the total SCAT2 score includes the Glasgow Coma Scale score and balance and coordination items; it ranges from 0 to 100, with any symptom or affection decreasing the score. However, in later revisions of the scale, the total SCAT2 score was considered a poor indicator and its use was discontinued.[39]

Outcome assessment

The symptom checklist in SCAT2 was used to compute a dichotomous variable—symptomatic or asymptomatic— at the two follow-up points. The first visit was scheduled within the first 2 weeks and was performed on average 5.9 days after mTBI (median 5; min: 1, max: 13 days). The second follow-up was scheduled at 3 months after mTBI and was

performed on average at day 106.4 (median 104; min: 78, max: 134 days). Patients were defined as symptomatic at any given point by using two different criteria: (i) when endorsing 3 or more symptoms, independently of their severity and (ii) when endorsing 10 or more symptoms, regardless their severity. The cut-off score for model (ii) was produced empirically, by analyzing the pattern of endorsement of concussion-like symptoms in the control group and finding that the 97.5 percentile score was 10.5 symptoms, out of a total of 22. The more liberal cut-off score was used for consistency with previous literature that shows, on one hand, frequent endorsement of up to 3 symptoms in non-concussed, healthy individuals[40,41] and, on the other hand, the elevated percentage of patients which do not completely recover to their preinjury status.[42]

Serum sampling for biomarkers

In all participants, as early as possible after injury, a single 4 mL blood sample was drawn in vacutainers with a separator gel for serum (#454058, VACUETTE® Z Serum Sep Clot Activator, Greiner Bio-One GmbH, Austria). Within 30 to 60 min of extraction, the sampling tube was centrifuged at room temperature at 4000 rpm for 10 minutes. The supernatant was stored in 300 µL aliquots at -80°C until analysis. Repeated freeze-thaw cycles were avoided. The levels of the proteins of interest were determined either by enzyme-linked immunosorbent assay (ELISA) commercial kits or by multiplex assays, using the MAGPIX system with the MILLIPLEX® Analyst 3.5 software (EMD Millipore Corporation, Billerica, MA, USA). The dilutions applied, the levels of detection and other characteristics of these kits are resumed in **Table 1**. Assay results were not available to the clinical personnel and were not used to guide treatment. Technicians were blinded to clinical data and CT results. All serum concentrations are reported in the measurement units originally recommended for each BM assay.

Statistical analysis

Descriptive statistics were obtained for each variable. Mean and standard deviation were used to describe continuous variables with normal distribution and the median, maximum, and minimum values for the continuous variables that were not normally distributed. The Shapiro-Wilk test and inverse probability plot were used to test whether data followed a normal distribution. Percentages and sample sizes were used to summarize categorical variables. To compare between-group differences (in categorical variables) χ^2 statistics or the Fisher exact test were used as appropriate. Between-group differences were determined by an independent

2-sample *t*-test or the Mann–Whitney *U* test, depending on statistical distribution. To correlate 2 continuous variables, the most conservative Kendall tau (when data did not follow a normal distribution) or Pearson correlation test (for data following a normal distribution) was used. Unless otherwise specified, differences were considered statistically significant when $p < 0.05$. For classification purposes, the area under the receiver operating curve (AUC) was determined with 95% confidence intervals (CIs). Statistical analyses were carried out with R v3.6.1[43] and the integrated development environment R Studio v1.2.315 (RStudio, Inc., Boston, MA, USA; http://www.rstudio.com). The following R packages were used in the analysis: XLConnect 0.2.15, gmodels 2.18.1, dplyr 0.8.1, rcompanion 2.1.7, referenceInterval 1.1.1, caret 6.0.82, and partykit.

Range of normality for BMs and management of nondetects. When using multiple assays for BMs, the management of values below the detection limit of the assay (nondetects) should be specified to avoid bias. In our study, when <15% of data were left-censored we substituted the nondetects by the assay lower limit of detection divided by two as recommended by the US Environmental Protection Agency.[44] When 15% or more data in any BM was left-censored, our procedure for managing nondetects was conducted according to the recommendations of Helsel.[44,45] To calculate the reference intervals (RIs) for BMs in serum, as well as their upper and lower values, the first step was to apply Horn's algorithm (implemented in the R package 'referenceIntervals'[46] to detect outliers.[47] Each detected outlier was reviewed, and, if the patient or the data were considered doubtful, the case was eliminated from the RI calculations. To calculate the upper serum RI limit, we used the distribution-free nonparametric method described in the Clinical and Laboratory Standards Institute guidelines C28-A3 for estimating percentile intervals[48,49] by using the R package 'referenceIntervals'.[46] In our data, we had multiple readings that were under the detection limit.

Logistic regression model. We used multiple logistic regression (MLR) to explore the associations between the dichotomized outcome variable—presence or absence of at least 10 or 3 symptoms irrespectively of severity— with continuous, ordinal, and categorical predictors. This analysis was conducted with binary logistic regression modelling. Preselected input variables were introduced in the model according to the method suggested by Hosmer et al.[50] Our goal was to obtain the best fitting model while minimizing the number of parameters.[50] In brief, risk factors in a continuous scale for the predefined outcomes were first separately tested by univariate analysis. Categorical variables were tested for significance via a

standard contingency table analysis of the outcome (y=0, 1) versus the k levels of the independent variable. Significance was tested with the Pearson chi-square test. All variables with p <0.25 in the univariate analysis were then entered in a MLR analysis.[50] Variables that were not statistically significant at p <0.05 were eliminated and a new model was generated without them. If none of the variables included in the first model was statistically significant, a backward elimination selection algorithm was used, based on maximising the likelihood ratio, and the best fitting model obtained from this was used for the following stage. In the third step, variables excluded in the univariate analysis were added individually to the final model to test statistical significance. According to Hosmer, this step is crucial for identifying variables that by themselves were not significantly related to the outcome, but could be important contributors to the final model in the presence of other variables.[50] In the final model, the original coefficients, their statistical significance, the 95% confidence intervals (CI), and the odds ratio (OR) were reported. A 2-tailed p value <0.05 was considered statistically significant for the MLR. Nagelkerke pseudo R-squared values were used as a goodness-of-fit measurement for the final model. Pseudo R-squared values range from 0 to 1, with higher values indicating a better model fit.

Conditional inference trees. In addition to the conventional logistic regression method, we used the URP-CTREE technique developed by Hothorn et al.[51] This method is an unbiased recursive partitioning tree-structured regression tool that identifies homogeneous subgroups from within an initial heterogeneous population. To conduct this analysis, we used the ctree function implemented in the partykit R package, a toolkit for representing, summarizing, and visualizing tree-structured regression and classification models.[52] In brief, ctree performs an exhaustive search of all possible splits of the input variables and selects the covariates that show the best split.[51] R code is available upon request from the corresponding author.

The accuracy of the 2 models was evaluated internally using both a training and an evaluation split for the original database. We used the following metrics: 1) confusion matrices with accuracy, sensitivity, specificity, and negative and positive predictive values; 2) the calculated area under the curve (AUC); and 3) the root-mean-squared error for evaluating the difference between the predicted values by a model and the observed values.

RESULTS

Participants

For a clear understanding of the selection, during the first 12 months of the study, the screening process was thoroughly documented. In that timeframe, from a consecutive series of 1144 mTBI patients, only 41 participants (3.6%) were recruited. Six-hundred thirty-eight patients (55%) were excluded for being outside of the age range and another 149 cases (13%) because they had arrived at the hospital later than 24 h following mTBI. Out of the remaining 367 patients between 18 and 65 years old that were admitted in the first 24 h of their mTBI, 158 presented with clinically relevant medical history. Other exclusion criteria resulted in 75 eligible candidates for the study, out of which 34 declined participation. Therefore, and because of the strict enrolment criteria, less than 4% of all presenting mTBI cases were included in this study. For funding reasons, the screening registry was discontinued for the rest of the recruitment period. In the remaining months, 54 additional patients were enrolled. Of the total 95 patients who were recruited, 13 were excluded from this analysis. Three eventually required a longer hospital stay for their fractures and were admitted for several days. Seven additional patients were excluded for failed blood extraction or serum separation; and 3, because incomplete SCAT assessment. A summary of the included/excluded cases is shown in **Figure 1**.

The final mTBI group had 82 patients. Fifty-two (63.4%) were men, with a median age of 33 years (min: 18, max: 64 years). The socio-demographic characteristics of the patients and controls are exposed in **Table 2.** Most patients presented with a GCS score of 15 (93.9%) and 56 had experienced LOC (68.3%). In all cases, the LOC duration was less than 5 minutes. Three (<1%) had seizures and 16 (19.5%) had vomited either before reaching the ED facility or during admission. The most frequent causes of injury were road traffic accident (42%) and sport activities (19.5%).

In the control group we enrolled 60 participants, of similar age, sex distribution and level of education as the mTBI patients. This cohort has been presented in detail elsewhere and is summarized in **Table 2.**[10] Some information regarding the level of education and BMI was not obtained. In the control group, forty participants (71.4%) did not show any abnormality in MRI. Incidental findings of no clinical relevance were reported in 16 cases (28.6%), most of which were unspecific foci of T2/FLAIR signal abnormality. All cases were thoroughly revised

and lacked any medical condition that would justify their exclusion, as has been described previously.[10]

Attrition

Fifteen patients (18.3%) were lost for all follow-up sessions. Complete follow-up was conducted in 65 patients at 1-2 weeks after mTBI (79.3%), and in 43 patients at 3 months (52.4%). Patients that attended the evaluation at 1-2 weeks after mTBI were not different from the complete cohort in terms of age, sex, acute number nor severity of concussion symptoms and ISS. However, there were several statistically significant differences between the group of patients which presented themselves for the second follow-up and the group of patients that did not attend. Women were less likely to attend the 3-months follow-up (Chi-square test, p = 0.023). Also, patients with a lower level of education were disproportionately absent at the session (p = 0.02, d = 0.58). Paradoxically, patients that presented with a higher number and more severe acute symptoms were also less like to attend that visit (U Mann-Whitney, p = 0.031 and 0.023, respectively). Finally, 3-months attendees did not differ by age, sex or general injury severity from the rest of the patients recruited.

Clinical symptoms at admission and follow-up

As early as admission and always within the first 24 h following mTBI, all patients were examined for symptoms and signs of concussion with the SCAT2. Headache was by far the most frequent acute symptom (85%), followed by a symptom reflecting a general discomfort – "Don't feel quite right"— in 67.5%. The endorsement of the most ten most frequent post-concussion symptoms recorded in the SCAT2 checklist, at the initial evaluation is depicted in **Table 3**. Concerning the general cognitive state, patients achieved in the early hours following mTBI a median SAC score of 26 (min: 17, max: 30).

At the first follow-up assessment, 1-2 weeks later, patients exhibited more post-concussion symptoms (p<.001, d=.99) and more severe p= d=1.05). Although lacking any type of specificity to brain injury, the item "Don't feel quite right" was also frequently endorsed by patients at the 1-week evaluation (52.3%), while less than 10% of the volunteers reported it (5 cases). However, the most frequently reported symptom at 1 week postinjury was neck pain (60.3%).

By 3 months following mTBI, we observed no difference in terms of symptom number or severity between patients and controls (**Fig. 2**). Neck pain was still present in nearly a third of the patients (30.2%). This somatic symptom together with all the items in the cluster of fatigue were the most frequently endorsed symptoms at the last follow-up examination; more than 20% of patients reported low energy, drowsiness, and/or trouble falling asleep. In fact, fatigue was reported by more than half of the attendees at both first two assessments, while having been a complaint for 35% of the healthy participants.

Serum biomarkers levels at baseline

Control participants: The number of valid samples, nondetects and the reference intervals for the 5 BMs assayed in the 60 participants of the control group are shown in **Table 4**.

mTBI patients: Serum samples were drawn at a median of 6.7 hours since injury (min: 2.1 h max: 24 h). In 34 patients (41.5%) serum samples were obtained within 6 h of injury. Casp-1 and IL-1β were below the lower limit of detection in all patients and controls. Even with the use of a second ultrasensitive kit for the IL-1β (HCYTOMAG-60K, EMD Millipore Corporation) we did not get any result after the failed attempt with a regular kit. In an initial univariate analysis, the mTBI group exhibited statistically significant increased levels of S100β, VEGF-A and CRP in comparison with the control group (**Table 5**). Logistic regression was used to determine AUROC curve for all three BMs together. In the MLR model including the 3 BMs none showed a statistically significant difference to separate patients from controls. Individually, only leptin showed a discrete ability to discriminate both groups. Leptin had a sensitivity of only 21% and a specificity of 91%. The AUROC was 0.57 and therefore leptin's discriminative value in mTBI is poor.

Outcome at 3-months following mTBI

Consequently, the mTBI patients were classified as displaying PPCS according to the two criteria described previously: (1) with the empirical criterion, i.e. with a cut-off of 10 symptoms; (2) with the "3-symptom or more" threshold. With the first criterion,.6 patients (14.3%) were classified as displaying clinically relevant post-concussion symptoms. With the second criterion, 19 patients (45.2%) were considered symptomatic at 3-months. These dichotomic outcome variables were used in the logistic regression analysis, in search for possible predictor factors.

Factor selection and MLR

The following variables were considered potentially altering the presence of PPCS in the mTBI group and were analysed as previously explained: age, sex, level of education, GCS score, LOC, PTA, having experiences seizures or, independently, vomiting in the first hours after mTBI, the ISS score, the number of symptoms displayed in the first 24h following mTBI and their severity, and the each BMs of the panel of interest

(in preparation)

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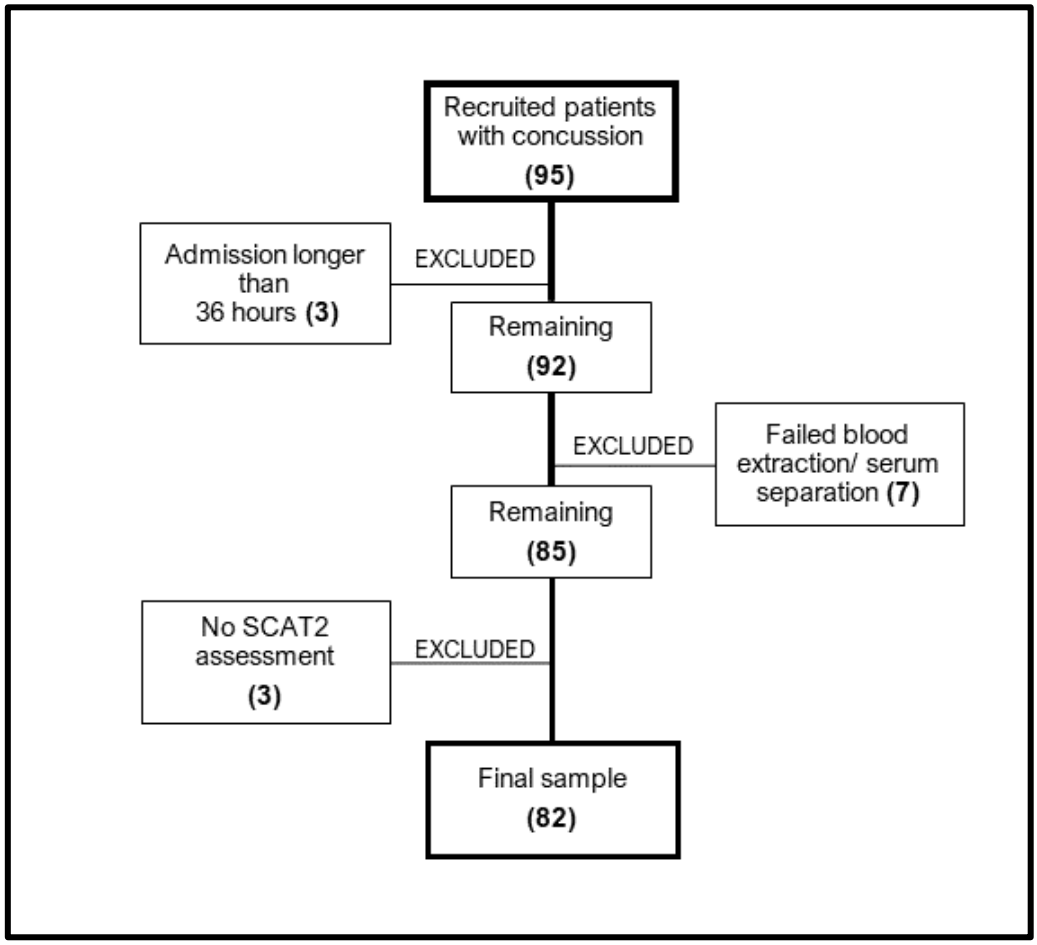

Fig. 1. Patient flow.

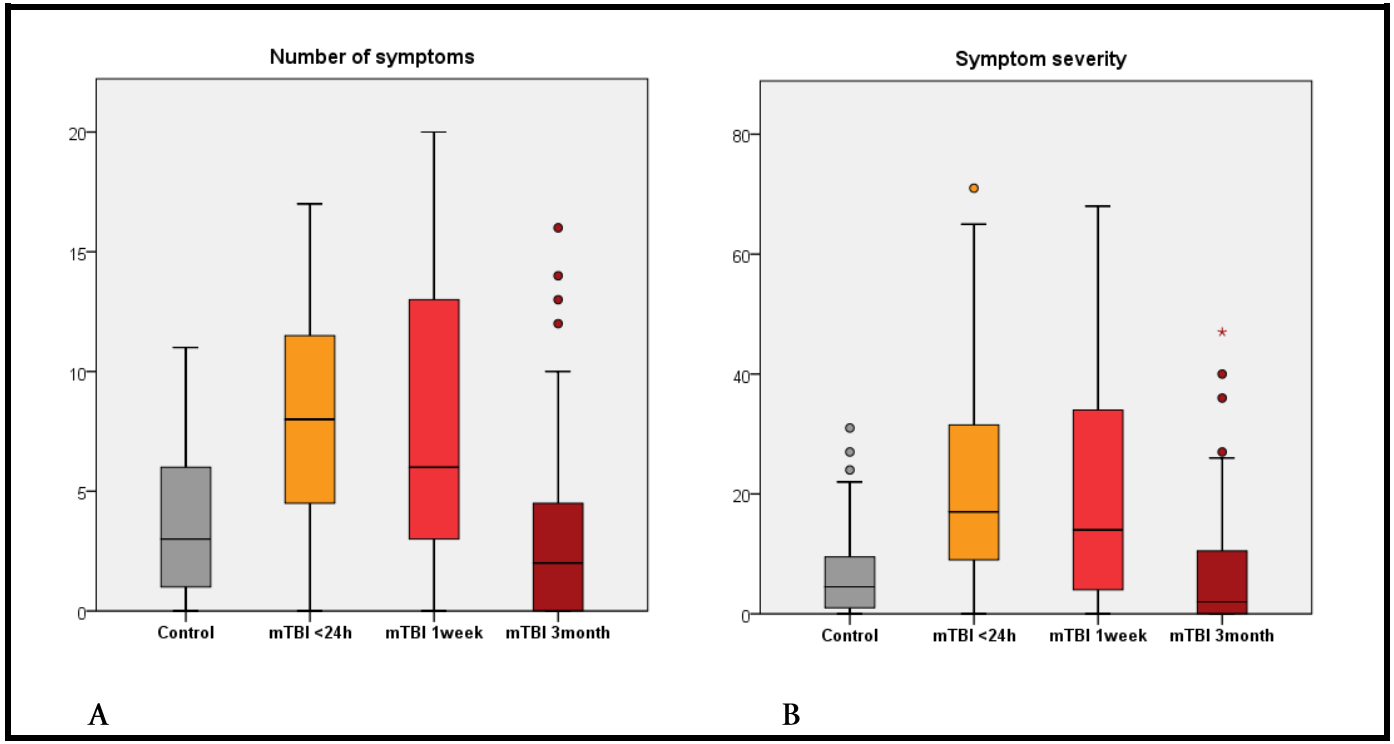

Fig. 2 Boxplots for the number of symptoms (A) and the total symptom severity (B), for the control group and the patients with mTBI, at the specified timepoints since injury. Data correspond to the following sample sizes: control group, n=60; mTBI at <24h, n=80, mTBI at 1 week, n=65, mTBI at 3 months, n=43. The horizontal line within the box designates the median of the variable of interest; first and third quartiles are designated by the bottom and top of the box, respectively. The upper and lower whiskers represent the maximum and minimum values below the upper fence (1.5IQR above the 75th percentile) or above the lower fence (1.5IQR below the 25th percentile), respectively. The colored circles and star represent individual outlier values. IQR: interquartile range; mTBI: mild traumatic brain injury.

Table 1. Technical characterization of the kits used for biomarker detection

Biomarker	Kit Type	Dilution	Detection range	Supplier	Reference
S100β	ELISA	1:1	2.7 – 2000 pg/mL	EMD Millipore Corporation	#EZHS100B-33K
VEGF-A	Multiplex	1:3	13.7 - 10,000 pg/mL	EMD Millipore Corporation	#HAGP1MAG-12K
Lep	Multiplex	1:3	137.2 - 100,000 pg/mL	EMD Millipore Corporation	#HAGP1MAG-12K
vWF	Multiplex	1:40,000	0.244 - 1,000 ng/mL	EMD Millipore Corporation	#HCVD3MAG-67K
CRP	Multiplex	1:40,000	0.012 - 50 ng/mL	EMD Millipore Corporation	#HCVD3MAG-67K
IL-1β	ELISA	1:1	3.2 – 10,000 pg/mL	eBioscience Inc	#BMS224HS
IL-1β	ELISA	1:1	0.16 - 10.0 pg/mL	EMD Millipore Corporation	#HCYTOMAG-60K
Casp-1	ELISA	1:1	12.5 - 800 pg/mL	Cusabio Biotech Co	#CSB-E13025h

Abbreviations: Casp-1: caspase 1; CRP: C-reactive protein; IL-1β: interleukin 1 beta; Lep: leptin; VEGF-A: vascular endothelial growth factor A; vWF: von Willebrand factor.

Table 2. Demographics and information concerning the injury

	mTBI group (n=82)	**control group (n=60)**	**p**
Sex (M:F)	52 : 30	38 : 22	[1]0.99
Age (years)	33 (18 - 65)	31.5 (18 - 64)	[2]0.96
Level of education (years)[a]	14 (4 - 22)	13 (8 - 22)	[2]0.90
Body mass index (kg/m^2)	25.1 ± 3.4	23.5 ± 3.0	[3]0.008
Underweight (<18.5)	1	4	[4]0.03
Normal weight (18.5 - 24.9)	32	41	
Overweight (25 - 29.9)	27	13	
Obese (>30)	4	1	
Not available	18	1	
Glasgow Coma Scale (15: 14)	77 : 5		
Loss of consciousness	56 (68.3%)		
Post-traumatic amnesia	55 (67.1%)		
Seizures	3 (3.7%)		
Main injury mechanism			
Acceleration - deceleration	31		
Direct blow	41		
Incidental fall	10		
Type of injury			
Assault	12		
Ground level fall/ Fall from height	8/2		
Road traffic accident:			
Vehicle-related/ Pedestrian	33/2		
Sports	16		
Work	1		
Other	8		
Time injury – extraction (h)	6.7 (2.1 - 24)		

[1] Chi-square, [2] Mann-Whitney, [3] Student's t-test, [4] Fischer's exact test;

[a] Not available in 15 cases in the mTBI group; mTBI: mild traumatic brain injury.

Table 3. The evolution of the most frequently endorsed symptoms in the first 24 hours following concussion and their incidence in the control group

	mTBI group		
	< 24 h (%)	**1 week (%)**	**3 months (%)**
Headache	85.0	49.2	16.3
"Don't feel right"	67.5	52.3	9.3
Fatigue or low energy	62.5	56.9	27.9
Neck Pain	58.8	55.4	34.9
"Pressure in head"	57.5	43.1	9.3
Feeling slowed down	51.3	53.8	14.0
Drowsiness	47.5	41.5	20.9
Feeling like "in a fog"	37.5	29.2	7.0
Dizziness	36.3	44.6	14.0
Difficulty concentrating	36.3	38.5	16.3

Table 4. Summary of the serum levels samples in the control group and the reference intervals

	Valid samples	**Nondetects**	**Lower limit**	**Upper limit**
S100β (pg/mL)	60	0	3.6	196.4
VEGF-A (pg/mL)	60	1	9.03	2289.0
Lep (pg/mL)	60	0	495.9	117221.8
vWF (ng/mL)	60	2	0.122	1372.0
CPR (ng/mL)	60	5	0.006	86800

CRP: C-reactive protein; Lep: leptin; VEGF-A: vascular endothelial growth factor A; vWF: von Willebrand factor.

Table 5. Detected levels of the biomarker panel in serum

	Control group			mTBI group			Mann-Whitney
	n	Median	Min - Max	n	Median	Min - Max	*p*
S100β (pg/mL)	60	27.9	1.31 – 358.36	82	42.09	5.51 – 364.17	0.007
VEGF-A (pg/mL)	60	292.5	6.85 - 2436	82	397	6.85 - 1816	0.037
Lep (pg/mL)	60	10181.5	467 - 126099	82	7544	68.6 – 69030	0.084
vWF (ng/mL)	60	18800	0.12 – 1851600	82	20246	0.12 – 1649200	0.690
CPR (ng/mL)	60	2448	0.01 - 165200	82	4174	0.01 - 1246400	0.045
Casp-1	Below detection range						
IL-1β	Below detection range						

the data in this analysis was rounded when below the limit of detection to LDL/2;

Casp-1: caspase 1; CRP: C-reactive protein; IL-1β: interleukin 1β; Lep: leptin; LDL: lower limit of detection; mTBI: mild traumatic brain injury; VEGF-A: vascular endothelial growth factor A; vWF: von Willebrand factor.

C. Longitudinal results of the neuropsychological examination

Between April 2014 and April 2017, in a first stage, 95 cases agreed to study participation and signed the written consent in the first 24h following mTBI, 3 for hospital admission due to polytrauma and another 3 for no available SCAT2 assessment. Finally, the study cohort for the concussion assessment included 89 patients rigorously selected by applying the criteria previously described. They were all scheduled to the neuropsychological assessment, but only 72 attended at least one of the two follow-up sessions.

Some of the most relevant characteristics of this subgroup are presented in **Table S-I**, which also includes the description of 60 participants that were included in the control group.

Table S-I. Sociodemographic indicators and clinical factors relevant to the mTBI subgroup of patients with mTBI that underwent neuropsychological evaluation and the control

	mTBI group (*n* = 72)	**Control group (*n* = 60)**	***p*[1]**
Sex (M:F)	51 : 21	39 : 21	0.47[2]
Age (years)	33 (18 - 64)	31 (18 - 64)	0.99
Education level (years)	14 (4 - 22)	13 (8 - 22)	0.80
Estimated Premorbid intelligence (TAP)	24 (9 - 30)	25 (17 - 30)	0.15
Loss of consciousness	51 (70.8%)		
Post-traumatic amnesia	50 (69.4%)		
Glasgow Coma Scale score (15: 14)	68 : 4		
Injury mechanism			
Acceleration - deceleration	28 (38.9%)		
Incidental fall	12 (16.7%)		
Direct impact	32 (44.4)		
Cause of injury			
Traffic accident	32 (44.4%)		
Sports-related accident	15 (20.8%)		
Assault	7 (9.7%)		
Ground-floor fall	10 (13.9%)		
Others	8 (11.1%)		

[1] Mann-Whitney, [2] Chi cuadrado; TAP: *Test de Acentuación de Palabras*.

Attrition

The neuropsychological evaluation could not be conducted in 17 patients, representing 19.1% of the complete cohort with mTBI, that failed to attend follow-up examinations. We

compared the group of patients that underwent neuropsychological evaluation with the rest of the cohort, and found no statistically significant difference between them, in terms of socio-demographic and clinical descriptors.

Performance validity and effort during cognitive testing

Traditionally, litigation is one of the most frequently named confounding factors in outcome assessment post-mTBI. This association has been highlighted by the literature published in the United States, where the culture of legal claims is substantially different than in most European countries. In some studies, societal costs related to litigation-related malingering are considered worrisome and proper strategies to identify feigned deficits are called for (Denning & Shura, 2017). However, the supposition that litigation is linked with intentional poor effort during neuropsychological assessment has been strongly questioned (Silver, 2012; Stulemeijer, Andriessen, Brauer, Vos, & Van Der Werf, 2007). Poor effort appears to be significantly linked with low education levels, fatigue and, in a lesser degree, with personality factors and not with litigation status (idem). Furthermore, litigation implicitly exposes the person to a system of legal representatives and healthcare providers that revolves around the importance of the injury and its deleterious effects, all of which increase the likelihood of nocebo effects (Vanderploeg et al., 2014). In unselected prospective samples, involvement in litigation and pursuing compensation were not related to neuropsychological functioning (Levin et al., 2013), reported symptoms (Kirkwood, Peterson, Connery, Baker, & Grubenhoff, 2014) or occurrence of depression or PTSD after TBI (Silver, 2012).

In our study, patients were informed that the results of the research-focused protocol do not substitute a forensic evaluation, if case they needed one. As a result, we chose not to control for litigation per se but directly for the risk of suboptimal effort made during testing. In our protocol, we used the first part of the Test of Memory Malingering (TOMM, Tombaugh et al., 2011). Although the original version requires two successive showings of the stimuli in distinct order, it was found that the application of only Part 1 of TOMM is equivalent or slightly more sensitive than the complete tool. The recommended cut-off score is 45 after the second trial in the test manual, or 40 after Part 1 (Denning & Shura, 2017). In our sample, all but three patients obtained after Part 1 a maximum (50) or nearly perfect score (49) and the minimum score achieved was 46, which is still comfortably above the thresholds in use. Because of this, the validity of the neuropsychological scores was not put under question and the factor of poor effort was not taken into consideration any further.

Addendum to the neuropsychological methodology

Statistical analysis in neuropsychological assessment is frequently affected by diminished statistical power due to a large number of variables used in the group comparisons. As previously

explained, most tests that are recommended for research in TBI by expert consensus are not sensitive to change after concussion. It has become clear that mTBI assessment should include novel tasks. However, designing an assessment battery that incorporates both traditional tests and tools with lesser clinical recognition inevitably increases the number of variables to be analyzed. In addition to applying multiple comparison corrections, various strategies have been proposed for minimizing the risk of type I error in neuropsychological data, such as principal component analysis (Levin et al., 2013), aggregated indices (Clarke, Genat, & Anderson, 2012) and other (Silverberg et al., 2017).

In this study, the neuropsychological variables have been assigned to four compounded indices of cognitive functioning: memory, attention, speed processing and executive functioning (**Table S-II)**.

In addition, the threshold for statistical significance was lowered from the routine p value of .05 and statistically significance was considered when $p < .005$. This decision was taken following recent suggestions by many authors to change the default p-value threshold for statistical significance from .05 to .005, in particular in pilot studies, like ours, and with small sample sizes, as the risk of reporting false positive results is higher (Benjamin et al., 2018; Ioannidis, 2018) .

All indices were formed by averaging the standard z-scores for the corresponding tests. The z scores were obtained either directly from the normative manual of the test or by transforming the T scores or scalar scores from the corresponding normative bibliography (Benedict, 1997; Casals-Coll et al., 2013; Peña-Casanova, 2009; Peña-Casanova, Quiñones-Úbeda, Gramunt-Fombuena, et al., 2009, 2009; Peña-Casanova, Quiñones-Úbeda, Quintana-Aparicio, et al., 2009; Rognoni et al., 2013; Strauss et al., 2006; Tamayo et al., 2012; Wechsler, 2004). The transformation controlled for age and sex, when available. However, no correction was performed per variable for the level of education, even if it was available, in an attempt to reduce the risk of overcorrection. For all aggregated cognitive indices, the potential effect of the variation in the level of education was examined in between-group analysis.

Indicators of executive dysfunction following mTBI

In addition to the classical tests that tap executive functioning and have been described previously, the protocol included 4 more tests that assess various supervisory processes: Wisconsin Card Sorting Task (WCST-CV4, Heaton, 2003), the Tower of London Dx (ToLDx, Culbertson & Zillmer, 2001), the Monetary Choice Questionnaire, also called the Delay Discounting Test (DDT, Kirby, Petry, & Bickel, 1999) and the Reading the Mind in the Eyes (RMiET, Baron-Cohen et al., 2001).

Table S-II. The composition of the aggregated cognitive indices. In the first column, there are the names of all the selected tests, and below each cognitive index the corresponding specific variables that were included.

Test	Memory index	Attention index	Processing speed index	Executive functions index
RAVLT	Total word number; Delay score (7th trial)			
BVMT-R	Total drawing score; Delay score			
TMT		Part A	Part A	Part B
WAIS-III		Digit forwards span; Digit Symbol Coding; Symbol Search	Digit Symbol Coding; Symbol Search	Digit backwards span; Letters and Numbers Sequencing;
Stroop Test			Word Reading; Color Naming	Word*Color Index
COWAT				Phonetic fluency index
CPT v5.2		Errors of omission	Hit reaction time	Errors of commission

BVMT-R: Brief Visual Memory Test- Revised; COWAT: Controlled oral word association test; CPT: Conners' Continuous Performance Test; RAVLT: Rey Auditory Visual Learning Test, TMT: Trail Memory Test; WAIS-III: Weschler Adult Intelligence Scale, the 3rd edition.

The WCST is a test designed to assess concept formation, set-shifting and perseverative behavior. Due to the nature of the test, it cannot be applied repeatedly. As no alternative versions are known, it was only included in the 3-months FU protocol. The decision was made to describe the longitudinal cognitive profile of patients with mTBI by using indices that were comparable in structure. Therefore, the WCST scores could not be included in the aggregated index for executive functioning.

ToLDx it is an excellent tool for planification and impulsivity assessment and ideally it should have been incorporated in the aggregated index for executive functioning. Nevertheless, due to logistical difficulties the test could not be used in the first year of the project. As a result, the scores for this test are missing in a considerably part of the cohort in comparison with the remaining battery. The scores in ToLDx are analyzed separately.

The DDT is a questionnaire thought to reflect on behavioral impulsivity and it was designed according to the devaluation reward theory. It has been shown that the subjective value a person assigns to a particular reward decreases as the time needed in order to achieve that reward increases. The indifference point corresponds to the moment when the delayed rewards is equally as valuable as the immediate incentive. The speed at which reward devalues with waiting can be a personality trait but it is also considered a marker of impulsive behavior, that can be modulated by TBI or acquired disorders. When showing a high indifference point, the reward´s value decreases exponentially, and the person chooses a smaller immediate reward over a "better" delayed choice in situations where others with a smaller indifference point would postpone gratification. The variables of interest in DDT scoring are the indifference points, *k*, for three categories of monetary amounts (small, medium and large).

Lastly, RMiET is a multi-choice questionnaire designed to assess theory of mind and mentalizing, abilities which are considered essential for social cognition. It has been extensively used in research concerning the autism spectrum disorder. Due to the vulnerability of orbitofrontal cortices in TBI, dysfunctions in interpersonal skills are often persistent sequelae of injury and are notoriously elusive to neuropsychological assessment. The use of this tool was aimed at explaining subtle deficits in social cognition that may appear post-concussion. It comprises 36 photographical items with pairs of eyes where the respondent must choose between four words the one that best described the emotional state inferred in the person in the picture.

Furthermore, the raw scores from two other tests, Trail Making Test (TMT) and Stroop, have been computed into interference measures. These interference indices could not be included in the aggregated index because no normative values are agreed upon in the literature on Spanish population, and their transformation to z-scores would have been debatable.

Block design, a substest of WAIS-III which is an indicator of visual reasoning and processing speed, was not included in the aggregated indices. In WAIS-III scoring there is no separation of the time bonification, therefore low values, especially at group level, have a poor specificity for cognitive functions.

Results on cognitive status following concussion

In **Table S-III**, the cognitive status of patients in the first 2 weeks following mTBI is detailed. All 4 aggregated indexes are inferior to the data in the control group, and memory displays a statistically significance impairment at the threshold of .005. The cognitive status, driven by the memory impairment but taken together with the other indices, also is below the average scores found in the control group, with a small effect size.

In addition, the prevalence of cases with scores above normality threshold in anxiety and depression, according to HADS, has been computed by grouping patients with scores between 8 and 10 (borderline) and above 11 (abnormal). Results show that there are significantly more patients who show symptoms of depression at 1 week after mTBI than in the control group, although the Fischer´s exact test does not reach statistical significance at $p < 0.005$.

For precaution, we examined the effect of both factors on memory levels in a general linear model, and being a patient at 1 week after an mTBI was confirmed as a statistically significant predictor ($F = 11.713$, $p = 0.001$, partial $\eta^2 = 0.104$) while having a HADS-D score exceeding the depression screening threshold was not ($F = 2.721$, $p = 0.101$). The same relation was observed regarding the global cognitive index (the group effect, $F = 2.497$, $p = 0.003$, partial $\eta^2 = 0.081$, and the category of HADS depression score, $F = 1.052$, $p = 0.307$).

At the 3-month FU assessment, the mTBI group displays similar levels of cognitive functioning with the control group. The mTBI and control groups did not differ in terms of age, sex distribution, education level or emotional distress prevalence ($p > 0.5$).

Results from the additional measures of executive functioning, not included in the aggregated indexes, are presented in **Table. S-IV**. In ToLDx, patients with mTBI required on average significantly longer initiation time. However, together with higher scores of the correct number of items they solved flawlessly, that result may not be an indicator of slower processing speed but of a better planification. In the control group, both number of correct items and initiation time are closer to the lower limit of the normality range (respectively, raw scores 4 and 41 seconds are both equivalent to a standard score of 96), while the same variables in the mTBI group are closer to the upper limit of the normality range (respectively, raw scores 7 and 74.5 second are equivalent to a standard score of 108). The improved scores achieved by the mTBI group at the 3-month evaluation could be due to practice effect, although a further recovery of their problem-solving abilities cannot be discarded.

Interestingly, the only scores which were not affected by test-retest effect in any way (WCST, due to a singular application) suggested that, 3 months following mTBI, patients could still present an alteration in their ability to tackle novel conceptual formation. The number of trials needed to complete the first category was statistically significantly higher than observed in the control group, but also considerably more variable (IQR 15 versus 1).

Table S-III. Longitudinal neuropsychological status of the group with mTBI based on the aggregated cognitive indices and emotional distress in comparison with the control group

	Control group (*n* = 60)		1-week FU (*n* = 70)				3-months FU (*n* = 46)		
Cognitive index	**Median (IQR)**	**Range**	**Median (IQR)**	**Range**	***p***	***r***	**Median (IQR)**	**Range**	***p***
Memory	0.73 (1.15)	-1.44–1.77	-0.13 (1.30) **	-2.55–1.78	.0002	.32	0.54 (0.99)	-1.77–1.86	.464
Attention	0.24 (0.75)	-0.92–1.60	0.06 (0.71)	-1.36–2.46	.035	.18	0.41 (0.68)	-0.99–2.25	.262
Processing speed	0.29 (0.67)	-0.78–1.71	0.01 (0.90)	-1.60–1.70	.034	.19	0.43 (0.81)	-1.19–1.61	.262
Executive fx.	0.11 (0.66)	-1.34–1.54	-0.01 (0.88)	-1,45–1.34	.034	.19	0.32 (0.68)	-0.75–1.63	.379
Global index	0.34 (0.70)	-0.96–1.50	0.01 (0.63)**	-1.64–1.44	.0004	.30	0.39 (0.57)	-0.69–1.35	.584
Emotional distress	**Yes / No**		**Yes / No**		***p***		**Yes / No**		***p***
HADS-A	14/45		19/50		.688		10/36		.905
HADS-D	2/57		12/57		.020		2/44		.764
Not available	1		1				0		

The values represent z scores. * $p < .005$; ** $p < .001$; *** $p < .0001$.

fx.: functions; *r*: effect size, IQR: interquartile range, FU: follow-up following mTBI; HADS-A: anxiety score dichotomized; HADS-D: depression score dichotomized; mTBI: mild traumatic brain injury

No impairment was observed on the scores of RMiET, Block design and interference indexes in TMT and Stroop.

The scores achieved by the repeated application of DDT are displayed in **Fig. S-1**. In comparison with the levels in the control group, the indifference point k differs significantly for small and medium amounts, at both points in time.

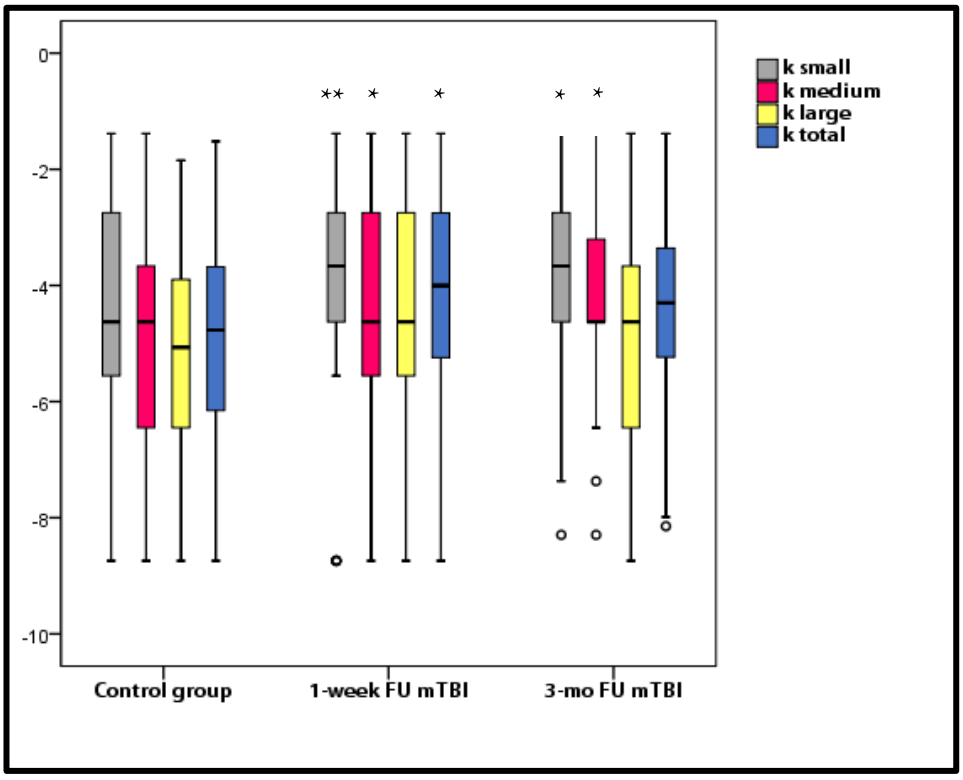

Fig. S-1. Delayed-discounting Rate (raw data subjected to logarithmic transformation) for the categories of amounts: small (25-35€), medium (55-65€) and large (75-85€). By comparison with the control values, *p < .05; ** p < .01.

Table S-IV. Longitudinal executive functioning of the group with mTBI in comparison with the control group, based on additional measures

	Control group			1-week FU				3-months FU			
	n	**Median (IQR)**	**Range**	**n**	**Median (IQR)**	**Range**	**p**	**n**	**Median (IQR)**	**Range**	**p**
Total correct items[1]	51	4 (4)	1–9	36	6 (4)	1–9	.049	33	**6 (4)***	0–10	**.003**
Total moves[1]	51	31(27)	2–78	36	24 (25)	1–67	.118	33	**19 (26)***	0–68	**.002**
Total initiation time[1]	51	41 (62)	11–170	36	**74.5 (50)****	**30–222**	**<.001**	33	**67 (59)***	28–252	**.002**
Total correct answers[2]	59	51 (10)	20–59	0				45	50 (13)	21–59	.233
Categories[2]	59	4 (2)	0–5	0				45	4 (3)	0–5	.267
Perseverative errors[2]	59	7 (4)	3–35	0				45	7 (6)	3–35	.298
Trials to 1st category[2]	59	11 (1)	10–65	0				45	**12 (15)***	10–65	**.002**
RMiET score	60	24 (5)	15–29	60	23(6)	8–30	.089	42	23 (5)	14–30	.573
TMT interference	59	1.2 (0.9)	0.3–3.5	59	1.3 (1.1)	0.4–3.3	.334	43	1.1 (0.80)	0.4–2.7	.168
Stroop interference	60	3.6 (7.1)	-32.4–20.2	65	2.4 (11.5)	-16.3–22.1	.540	42	3.5 (9.7)	-10.5–17.2	.940
Block design	60	50 (17)	14–67	64	50 (22)	16–66	.739	46	53.5 (17)	28–66	.112

The values represent raw scores. [1] Tower of London - DX; [2] Wisconsin Card Sorting Test; * $p<.005$; ** $p<.001$; *** $p<.0001$.
DDT: Delay Discounting Test IQR: interquartile range, FU: follow-up following mild traumatic brain injury. RMiET: Reading the Mind in the Eyes test; TMT: Trail Making Test. In Total moves, Perseverative errors and Trials to 1st category a higher score is indicative of a worse performance.

Endorsement of post-concussion symptoms and predicting PPCS

Data on symptom endorsement and other SCAT2 scores are included in **Table S-V.** As expected, patients reported significantly more and more intense symptoms in the first hours and days after mTBI, up to two weeks. Global cognitive functioning was impaired in the first 24 hours after concussion, and on average maintained a poorer score after a week ($p = 0.015$, not significant). During the ED assessment, mBESS could not be applied in a raised number of patients (23.3%). At the FU visits, the number of patients unable to perform the task reduced rapidly but the patients' score was never indicative of loss of balance, in comparison with the control values.

Regarding the prevalence of PPCS, we classified the sample according to the two criteria described previously (Supplementary Material, Section B). Nearly half the patients examined at the 3-month visit endorsed at least 3 symptoms and one out of seven reported ten or more symptoms. Although we reported the prevalence of patients fulfilling the same criteria at the 1-week assessment for presenting a complete picture of their evolution but labeling the symptoms as persistent so soon after concussion is unwarranted. We further focused on the PPCS displayed at 3 months.

For the examination of the variables that could be associated to PPCS, the statistical approach was described in detail in **Section B** (binary logistic regression models). In brief, aiming to identify the clinical variables that predict PPCS in this sample we examined socio-demographic descriptors (age, sex and highest level of education attained), clinical markers of mTBI severity (GCS, LOC, PTA) in addition to the number and severity of symptoms reported in the initial evaluation and the SAC score. Lastly, we examined the anxiety and depression scores referred at the same FU visit. The parameters of the most parsimonious models are depicted in **Table S-VI.**

Table S-V. Longitudinal data on concussion symptoms and other SCAT2 components, in comparison with the control group.

	Control group (*n* = 60)	< 24 h mTBI (*n* = 86)		1-week FU (*n* = 70)		3-months FU (*n* = 46)	
	Mdn (IQR, Range)	Mdn (IQR, Range)	*p*	Mdn (IQR, Range)	*p*	Mdn (IQR, Range)	*p*
Nº Symptoms	3 (5, 0-11)	8 (6, 0-17)	<.0001	6 (10, 0-20)	<.0001	2 (6, 0-16)	.27
Severity of Symptoms	4 (10, 0-31)	17 (22.5, 0-71)	<.0001	14 (30, 0-68)	<.0001	4.5 (14, 0-47)	.85
SAC	27 (4, 22-30)	26 (2, 17-30)	.004	26 (3, 17-29)	.015	28 (3, 22-30)	.43
mBESS[1]	24 (14-30)	24 (8, 0-30)	.876	22 (8.5, 10-30)	.058	23.5 (8, 20-30)	.70
SCAT2 global score[1]	87 (9, 73-97)	82 (9, 49-95)	<.0001	82 (14, 57-94)	<.0001	87 (11, 68-99)	.91
PPCS[2]**–criterion 1 (Yes)**	35 (58.3%)	-		52 (74.3%)		22 (47.8%)	
PPCS–criterion 2 (Yes)	2 (3.3%)	-		25 (35.7%)		7 (15.2%)	

[1]mBESS was not applied in 20 cases (23.3%) at < 24 h and 4 cases (5.7%) at the 1-week FU. As a result, SCAT2 global score could not be computed for the same cases.

[2]criterion 1: endorsing 3 or more symptoms; criterion 2: endorsing 10 or more symptoms.

FU: follow-up; IQR: interquartile range; mBESS: modified Balance Error Scoring System; SAC: Standardized Assessment of (cognition in) Concussion; SCAT2: Sport Concussion Assessment Tool 2nd Edition; SCAT: PPCS: persistent post-concussion symptoms.

Table S-VI. Binomial linear regression models for PPCS at 3-months after mTBI

	OR	*p*	CI (95%)	*p*-model	[1]*Pseudo-R²*
PPCS–criterion 1					
HADS anxiety	23.82	0.009	2.2–256.88	<0.001	0.49
Nº early symptoms	1.30	0.016	1.05–1.61		
PPCS–criterion 2					
HADS anxiety	33.00	0.001	3.16–343.96	<0.001	0.44

Models included intercept. [1] Nagelkerke.

The number of symptoms reported at the initial ED visit is a significant predictor for reporting 3 or more symptoms at 3 months following concussion. At first sight, the interpretation of the models would suggest that a patient that presents with an anxiety score above normality threshold in the HADS questionnaire has a risk of presenting PPCS 22 times higher than a patient with normal anxiety scores. This corresponded to the criterion of endorsing 3 or more PCS symptoms. For the empirical criteria (10 or more symptoms), the odds ratio for belonging to the subgroup with abnormal anxiety scores ascended to 32. However, the 95% confidence intervals are extremely wide and therefore the finding of such a strong relationship is doubtful. This is probably due to the small sample size and especially the small number of cases with PPCS (19 and 6, respectfully).

Overall discussion on the neuropsychological analysis

Attrition is one the most important limitations on the results of this study, including the evaluation of neuropsychological data. It affects generalizability and, in this sample, it has led to a small sample available for longitudinal analysis (n = 46 or smaller, if early variables were missing or procedures were not performed). The constraints of the sample size are noteworthy in examining the predictive value of anxiety symptoms for endorsing PPCS.

This neuropsychological analysis replicates and extends on the findings of the first study, were memory deficits were shown to have the highest effect size at 1-2 weeks following mTBI, in this sample. Due to the nature of the aggregated indices, these results do not allow the distinction between visual and verbal memory, or between learning and evocation processes.

The SCAT2 symptoms scores and the other subcomponents also replicate the findings of the early subsample. A distinction can be made regarding the interpretation of the SAC index, due to the change in the statistical significance threshold. Together these results add to previous studies that have shown SAC is sensible to post-concussion impairment and reflects its improvement over time.

The executive performance was similar in the mTBI group as control values, by nearly all additional indicators examined. The scores on The Monetary Questionnaire DDT were an exception and suggested the mTBI group on average chose immediate smaller rewards over delayed larger ones. In the general population, the indifference point k is independent of the magnitude of the economical reward per se, and depends on the magnitude of the increment and time. However, in our group the values of k have been more sensitive in suggesting impulsive behavior after mTBI with small and medium amounts of money, but not with large amounts. Although the differences in magnitude are not remarkable (25-35€ versus 75-85€), the effect is consistent. This has also been seen in a study on individuals with schizotypal personality disorder (Li et al., 2016). Because the devaluation function is expected to be equivalent for the three categories of amounts, many studies report only the *k* total. The hypothesis of a more impulsive behavior in response to smaller monetary sums, but not larger ones, in situations of mild disinhibition, should be explored in future studies. On the other hand, the DDT results could reveal differences in personality traits that we did not account for.

The protocol selected for this thesis combined traditional neuropsychological tests and more novel tools. However, the protocol did not reveal markers of persistent cognitive impairment. As discussed previously, there are many factors that could explain the neuropsychological profile observed in this sample. First, it is likely that most cases of post-concussion cognitive dysfunctions that were apparent at the 1-week FU resolved by 3 months. Second, we must acknowledge the limitation of a cumulative approach as aggregated indexes. Any marginal dysfunctions, that would have affected a minority of cases or specific to particular variables, are less likely to be observed with this methodology.

Moreover, functional MRI studies have showed that in some cases, despite normal neuropsychological results, the brain expenditure of energy is increased, as an abnormal allocation of resources takes place to sustain cognitive functioning at normal parameters (McAllister et al., 2001). This could explain the PPCS, and in particular cognitive PPCS, regardless of neuropsychological scores.

In addition, future studies on concussion neuropsychological assessment, due to the supposition that cognitive impairment is subtle and can be very heterogenous in function, could also benefit from including tests based on Item-Response Theory, as the NIH Cognition Toolbox (Holdnack et al., 2017). Adjusting the difficulty of the task to the performance elicited by the patient can improve twofold the accuracy of assessment: the scores achieve a higher granularity in less time, and therefore it is possible to apply more tests with less risk of cognitive fatigue.

D. Genetic vulnerability for an incomplete recovery following mTBI

A growing body of literature has been focusing on genetic factors that play a role in the pathophysiology of TBI, and their usefulness in explaining outcome variation (Jordan, 2007; McAllister, 2015). Multiple polymorphisms that have been linked to neuropsychological functioning have been studied in relation with the cognitive recovery following TBI. The rationale for the genetic analysis performed here is presented in the **Introduction** of this thesis (**section 6.4**), and considerations on the procedures employed previous to this point are included in the **Participants and Methods**, **section 3.4**.

Addendum to the methodology of the genetic data

Genotype frequencies for all selected polymorphisms were tested for Hardy–Weinberg equilibrium (HWE) with the chi-squared test, by employing a software available online (Rodriguez, Gaunt, & Day, 2009). The distributions did not differ from HWE expectations, in the control group nor in the subsamples of the mTBI group (as they presented at the two FU visits).

For further statistical analysis, all polymorphisms were dichotomized.

The minor allele T of the ANKK1 rs1800497 corresponds to the TaqIA (or Taq1A) polymorphism of the dopamine D2 receptor DRD2 gene and represents what was known as the DRD2*A1 allele, whereas rs1800497(C) represents the DRD2*A2 allele. However, this nomenclature is obsolete after it was established that rs1800497 does not belong to the DRD2 gene. Extensive research in psychiatric disorders and addiction has linked the T allele with a more dysfunctional behavior. For comparison purposes, alleles of ANKK1 rs1800497 were classified as follows: C/T or T/T alleles were grouped as T+, and the homozygous C/C was labelled T-. The procedure is consistent with McAllister et al., 2008.

Genotypes of two SNPs (rs7412 and rs429358) are required to reconstruct the ApoE allelic variant. ApoE alleles were determined as ε2 [rs7412(T)/rs429358(T)], ε3 [rs7412(C)/ rs429358(T)], or ε4 [rs7412(C)/rs429358(C)]. For the evaluation of a potential detrimental effect of the ApoE-ε4 allele, patients with ε2/ε2, ε2/ε3, and ε2/ε3 genotypes were grouped as ε4-, and ε3/ε4 or ε4/ε4 genotypes were grouped as ε4+, as conducted in multiple previous studies (Teasdale et al., 1997; Yue et al., 2017).

The BDNF rs6265 and COMT rs4680 alleles both involve a G-to-A substitution, which results in the replacement of the valine by methionine (Met) in the BDNF and COMT expressed proteins. The participants were separated based on the presence or absence of Met in the expressed protein. As such, individuals with G/G homozygous forms produce only the Val-containing proteins and have been labeled Met-, whereas carriers of G/A or A/A have been analyzed together as the Met+ group (Krueger et al., 2011; Winkler et al., 2017).

The SNP rs3219119 of the PARP-1 gene involves an A to T substitution, which hypothetically results in distinct PARP-1 enzymatic efficiency (Sarnaik et al., 2010). Because in previous studies the homozygous allele (A/A) has been associated with a favorable outcome and a better cognitive performance following TBI (Nielson et al., 2017; Sarnaik et al., 2010), we have labelled the A/T and T/T alleles as T+, and the A/A as T-.

Results

The determination of the selected polymorphisms for genes ApoE, ANKK1, BDNF, COMT and PARP-1 was completed in 56 healthy volunteers and 87 patients from the mTBI group, 70 of which attended neuropsychological FU. The cognitive indexes are presented for 68 patients at 1-2 weeks following mTBI, and for 44 patients at 3 months post-injury.

Table S-VII displays the allelic distribution of the selected genes, in the mTBI patients where neuropsychological data was available and the control sample. Age, sex and education levels were compared between participants with and without the presumed risk genetic expression for each individual gene, separately for patients and healthy participants. In the control group, these factors did not differ with statistical significance. However, concerning the polymorphism ANKK1 in the mTBI group, patients in the T+ group were younger than in the T- (Mann-Whitney, U = 345.5, $p = 0.018$). Furthermore, women were disproportionately present in the Val/Val group of the COMT gene, which is putatively the group associated with a poorer outcome (Fisher's exact test, $p = 0.041$). The frequency of two clinical indicators of mTBI severity, i.e. GCS score and LOC, is also presented. All distributions were compared with Fisher's exact test and were found not statistically significant.

Table S-VII. Sociodemographic and clinical description by genotype.

Gene	Genotype	Age[1]	Sex (F:M)	Level of education[1]	GCS (15/14)	LOC (Y/N)
Control group (n=56)						
ANKK1	T- (n=35)	33(26), 18-64	11:24	13(6), 8-20	-	-
	T+ (n=21)	29 (24), 18-58	7:14	13(4), 8-22	-	-
ApoE	ε4+ (n=13)	30 (33), 18-64	3:10	13 (6), 8-18	-	-
	ε4- (n=43)	32 (19), 18-58	15:28	13 (5), 8-22	-	-
BDNF	Val/Val (n=37)	31 (20), 18-63	11:26	13(4), 8-22	-	-
	Met+ (n=19)	42 (26), 18-64	7:12	12 (4), 8-20	-	-
COMT	Val/Val (n=13)	27(22), 18-63	5:8	12(4), 8-18	-	-
	Met+ (n=43)	34(25), 18-64	13:30	13(4), 8-22	-	-
PARP-1	T- (n=19)	31(28), 18-57	7:12	13(6), 8-20	-	-
	T+(n=37)	32 (23), 18-64	11:26	13(4), 8-22	-	-
mTBI group (n=70)						
ANKK1	T- (n=45)	38.5 (20), 18-61	13:32	13 (4), 6-22	40/5	28/17
	T+(n=25)	28.5 (18), 18-47	8:17	14 (4), 4-22	25/0	19/6
ApoE	ε4+ (n=12)	29 (17), 18-46	2:10	13.5 (4), 4-22	12/0	7/5
	ε4- (n=58)	34.5 (20), 18-61	19:39	13 (4), 8-19	53/5	38/20
BDNF	Val/Val (n=49)	34 (17), 18-61	15:34	13(4), 6-22	47/2	30/19
	Met+ (n=21)	30 (26), 18-60	6:15	14 (4), 4-22	18/3	15/6
COMT	Val/Val (n=20)	33 (18), 20-52	9:11	15 (4), 6-18	18/2	10/10
	Met+ (n=50)	33(21), 18-61	12:38	13 (4), 4-22	47/3	35/15
PARP-1	T- (n=27)	32 (20), 18-61	7:20	15 (8-20)	26/1	19/8
	T+ (n=43)	33 (20), 18-53	14:29	13 (4), 4-22	39/4	26/17

[1] Age and the level of education are presented as Median (IQR), min-max.

ApoE: apolipoprotein E; ANKK1, ankyrin repeat and kinase domain containing 1; BDNF: brain-derived neurotrophic factor; COMT: catechol-o-methyltransferase; FU: follow-up; GCS: Glasgow Coma Scale score; LOC: loss of consciousness; mTBI: mild traumatic brain injury; M: mean; PARP-1: poly(ADP-ribose) polymerase; SD: standard deviation; Y/N: yes/no.

Genotype association with cognitive functioning

Multiple comparisons were performed with the domain-specific cognitive indices and the global cognition index based on the pre-determined polymorphic groups described previously. Results are presented in **Tables S-VIII and S-IX.** In most cases, no differences were observed by allelic group. In addition, as the smallest p-value was 0.014, by applying any strategy of correction for multiple comparisons would render all observed differences not statistically significant (corrected p-value 0.01 per cognitive index).

However, the results suggest that ANKK1 and PARP-1 groups could affect the memory function post-mTBI. At 1 week after mTBI, the carriers of the T allele of the ANKK1 appear to have lower scores on the memory tests. That was also observed at the 3-month evaluation, where lower scores in memory function were also found in the T+ PARP subgroup. Therefore, these possible associations were explored further.

In linear regression models, controlling for the level of education, being in the ANKK1 T+ group conferred a statistically significant disadvantage in memory function at 1-week after mTBI (Wald's chi-squared = 7.748, p = 0.005) that was also found at 3-months after mTBI (Wald's chi-squared = 6.315, p = 0.012). The interaction between ANKK1 variant and age (due to the difference in this variable between ANKK1 subgroups) was tested, but it was not statistically significant. Furthermore, controlling for the level of education, carrying a T- allele of the PARP-1 gene was associated with a reduction in memory performance at 3 months after mTBI (Wald's chi-squared = 8.266, p = 0.004). This was not visible at 1-week after mTBI (Wald's chi-squared = 1.246, p = 0.264).

According to Akaike information criterion, the best models explaining memory aggregated index in this sample included the level of education, the ANKK1 status and the severity of the inital concussion symptoms, assessed in the first 24 hours. The PARP-1 polymorphism, age, sex, GCS, LOC and emotional distress (reported in HADS on the day of the memory examination) were examined but did not prove statistically significant useful factors in the model. The parameters of the models are displayed in **Table S-IX**. Both models have low-to-moderate indicators of goodness of fit (R^2), leaving more than half of the variation in the variable of interest unaccounted for. Every additional year of formal education attained is consistently associated with an increment of about one decimal in memory function standardized score (0.095). This effect was observed at both assessments. Furthermore, every six additional points in the severity score of the symptoms reported in the first 24 hours following mTBI reduce the memory score by nearly one decimal point (0.09 – 0.10 in the *z*-score of the aggregated index). Six points is the maximum intensity score for any symptom in SCAT2, so two moderate symptoms or one very severe symptom could have this effect. Lastly, being a carrier of the T allele has an independent effect and is associated with a reduction in memory scores of 0.5 SD, which was found at both FU timepoints. However, the variation of this indicator is considerable as the 95% confidence interval of the decrement ranges between 0.1 and 0.9 SD.

The genetic variants of interest were also analyzed in relation with the presence of PPCS, as classified by the two criteria described previously. Models of binomial logistic regression were designed and none of the polymorphisms was associated with PPCS in a statistically significant manner.

Table S-VII. Cognitive differences following mTBI observed at the 1-week FU assessment between groups with different genetic constitution.

Gene	Genotype	Memory		Attention		Speed processing		Executive functioning		Global index	
		M ± SD	*p*	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*
ANKK1	**T- (n=44)**	0.33 ± 0.98	**0.027**	0.12 ± 0.54	0.376	0.20 ± 0.68	0.058	0.07 ± 0.55	0.174	0.12 ± 0.52	0.065
	T+(n=24)	-0.48 ± 0.94		-0.04 ± 0.71		-0.15 ± 0.62		-0.14 ± 0.66		-0.20 ± 0.55	
ApoE	**ε4+ (n=12)**	-0.09 ± 1.38	0.641	0.05 ± 0.65	0.596	0.21 ± 0.68	0.334	-0.11 ± 0.60	0.688	0.01 ± 0.73	0.584
	ε4- (n=56)	-0.11 ± 0.91		0.06 ± 0.60		0.05 ± 0.68		0.23 ± 0.60		0.01 ± 0.51	
BDNF	**Val/Val (n=47)**	-0.14 ± 1.01	0.705	0.10 ± 0.61	0.132	0.15 ± 0.67	0.053	-0.06 ± 0.59	0.268	0.01 ± 0.55	0.619
	Met+ (n=21)	-0.02 ± 0.99		-0.03 ± 0.61		-0.09 ± 0.67		0.12 ± 0.61		-0.01 ± 0.57	
COMT	**Val/Val (n=19)**	0.03 ±1.09	0.393	-0.06 ± 0.72	0.232	0.09 ± 0.76	0.886	-0.07 ± 0.63	0.608	-0.01 ± 0.65	0.973
	Met+ (n=49)	-0.16 ± 0.97		0.11 ± 0.56		0.07 ± 0.65		0.03 ± 0.58		0.13 ± 0.51	
PARP-1	**T- (n=27)**	0.13 ± 0.93	0.083	0.12 ± 0.47	0.212	0.22 ± 0.65	0.174	0.09 ± 0.54	0.363	0.14 ± 0.51	**0.048**
	T+ (n=41)	-0.26 ± 1.02		0.22 ± 0.68		-0.14 ± 0.69		-0.06 ± 0.63		-0.79 ± 0.56	

Uncorrected *p* values.

ApoE: apolipoprotein E; ANKK1, ankyrin repeat and kinase domain containing 1; BDNF: brain-derived neurotrophic factor; COMT: catechol-o-methyltransferase; FU: follow-up; M: mean; mTBI: mild traumatic brain injury; PARP-1: poly(ADP-ribose) polymerase; SD: standard deviation.

Table S-VIII. Cognitive differences following mTBI observed at the 3-month FU assessment between groups with different genetic constitution.

Gene	Genotype	Memory		Attention		Speed processing		Executive functioning		Global index	
		M ± SD	*p*	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*	M ± SD	*p*
ANKK1	**T- (n=28)**	0.61 ± 0.71	**0.017**	0.40 ± 0.68	0.566	0.45 ± 0.57	0.354	0.38 ± 0.56	0.341	0.46 ± 0.49	0.118
	T+(n=16)	0.24 ± 0.79		0.31 ± 0.51		0.27 ± 0.51		0.16 ± 0.46		0.19 ± 0.42	
ApoE	**ε4+ (n=6)**	0.23 ± 1.27	0.803	0.32 ± 0.42	0.855	0.37 ± 0.35	0.907	0.03 ± 0.48	0.289	0.25 ± 0.58	0.559
	ε4- (n=38)	0.42 ± 0.71		0.37 ± 0.65		0.39 ± 0.58		0.34 ± 0.53		0.38 ± 0.47	
BDNF	**Val/Val (n=31)**	0.38 ± 0.80	0.928	0.24 ± 0.58	0.072	0.36 ± 0.53	0.827	0.23 ± 0.51	0.185	0.30 ± 0.48	0.322
	Met+ (n=13)	0.44 ± 0.80		0.68 ± 0.63		0.45 ± 0.63		0.46 ± 0.57		0.51 ± 0.49	
COMT	**Val/Val (n=13)**	0.49 ± 0.85	0.403	0.47 ± 0.38	0.571	0.54 ± 0.49	0.433	0.36 ± 0.54	0.616	0.47 ± 0.47	0.252
	Met+ (n=31)	0.36 ± 0.77		0.32 ± 0.70		0.32 ± 0.57		0.27 ± 0.53		0.32 ± 0.49	
PARP-1	**T- (n=19)**	0.74 ± 0.49	**0.014**	0.34 ± 0.46	0.868	0.42 ± 0.57	0.731	0.36 ± 0.54	0.578	0.47 ± 0.42	0.281
	T+ (n=25)	0.14 ± 0.88		0.38 ± 0.73		0.36 ± 0.55		0.26 ± 0.53		0.28 ± 0.52	

Uncorrected *p* values.

ApoE: apolipoprotein E; ANKK1, ankyrin repeat and kinase domain containing 1; BDNF: brain-derived neurotrophic factor; COMT: catechol-o-methyltransferase; FU: follow-up; M: mean; mTBI: mild traumatic brain injury; PARP-1: poly(ADP-ribose) polymerase; SD: standard deviation.

Table S-IX. Linear regression models for memory function after mTBI

	Coefficient *b*	*p*	CI (95%)	*p*-model	R^2
Memory aggregated index at 1 week after mTBI					
Education level	0.095	0.001	0.031 – 0.158		
Severity of early symptoms	-0.015	0.012	-0.028 – -0.002	< 0.0001	0.379
ANKK1 T+	-0.537	0.005	-0.921 – -0.153		
Memory aggregated index at 3 months after mTBI					
Education level	0.095	0.009	0,014 – 0,177		
Severity of early symptoms	-0.017	0.021	-0,030 – -0.003	< 0.0001	0.431
ANKK1 T+	-0.497	0.014	-0,894 – -0,100		

Models included intercept.

Overall discussion of the genetic analysis

In this cohort, ANKK-1 polymorphism was the only genetic variant that was associated with post-concussion cognitive performance in a statistically significant manner. Following the initial report of McAllister et al. and the replication study conducted by the same group (Mcallister et al., 2008; McAllister et al., 2005) and the report from the multi-centric TRACK-TBI study (Yue et al., 2015), this represents the third report to confirm the ANKK-1 gene plays a role in mTBI cognitive recovery. Pharmacotherapies that target dopamine have consistently improved cognitive impairment following TBI, but the exact paths in which TBI induces dopamine dysregulation remain unidentified (Bales et al., 2009). Brain areas vulnerable to TBI, such as the frontal cortex and striatum, abundantly contain dopamine receptors and others, as the hippocampus, are functionally modulated by dopaminergic activity. After moderate and severe TBI, reduced dopamine transporter levels are most commonly seen in the caudate, supposedly reflecting the damage of the nigrostriatal tract produced by axonal injury and associated midbrain damage (Jenkins et al., 2018).

In several studies, carrying at least one ε4 allele of the ApoE gene was associated with a decreased memory performance at 6 months after mTBI (Yue et al., 2017) and other post-traumatic impairments. However, a recent systematic review concluded that the deleterious effect of the ε4 could be limited to recovery after severe TBI, as its contribution to the evolution after mTBI has not been robustly confirmed (Lawrence, Comper, Hutchison, & Sharma, 2015).

The selected polymorphism of COMT, another gene involved in dopamine availability and processing, was not associated with the neuropsychological status, as it was assessed by the

aggregated indexes. One study with military samples found that a straightforward association between the COMT Val158Met genotype and better functional outcome (higher 6-month GOSe) was not visible in the absence of PTSD (Winkler et al., 2017). Moreover, a recent study on a cohort of 223 patients with moderate and severe TBI also did not find evidence for an association between COMT status (Willmott, Withiel, Ponsford, & Burke, 2014).

In a study using topological data analysis on a sample of 586 TBI patients with 87.3% of cases with mild severity, PARP-1 was identified as a candidate predictor of general functional deficits after mild TBI (Nielson et al., 2017). In our study, none of the genetic variants examined was statistically significant associated with PPCS. In multiple regression models, the association of the PARP-1 with memory functioning was not established. However, it is worth mentioning that a statistically significant univariate relationship between PARP-1 and memory performance was found at 3 months but not at 1 week following concussion. This could suggest that the DNA-repairing mechanisms regulated by PARP-1 are part of the long-term processes that appear after concussion. An alternative hypothesis would be heterogeneity in the composition of the subsamples evaluated at the 2 FU visits in ways we did not account for.

Limitation of the genetic analysis are acknowledged. Several allelic frequencies have been shown to vary by race, and we have not collected this variable, therefore the potential effect of race on the distribution of the polymorphic variants has not been tests in this sample.

E. Conclusions (for the Supplementary Material)

1. Serum levels of S100b, vWF and VEGF-A are increased in the first 24 hours after concussion.

2. At 3 months post-trauma, the cognitive performance of the patient group was similar with the group of non-head injured participants. However, 47.8% of the patients endorsed 3 or more post-concussion symptoms. Displaying 3 or more PPCS at 3 months was associated with a greater burden of early symptoms.

3. Memory performance in the mTBI cohort is best explained by the level of education, severity of early symptoms and the ANKK1 polymorphism. Being a carrier of the T+ allele of the gene is associated on average with a 0.5 *SD* decrease in memory function after concussion.

F. References

G. References

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