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Reference:
Verduyn Carl, Bjerke Maria, Duerinck Johnny, Engelborghs Sebastiaan, Peers Koenraad, Versijpt Jan, D'haeseleer Miguel.- CSF and blood neurofilament levels in athletes participating in physical contact sports : a systematic review
Neurology / American Academy of Neurology - ISSN 0028-3878 - 96:15(2021), p. 705-715
Full text (Publisher's DOI): https://doi.org/10.1212/WNL.0000000000011750
To cite this reference: https://hdl.handle.net/10067/1757940151162165141

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Light chain neurofilament revealing brain tissue damage in physical contact sport athletes? A systematic review

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Word count: Introduction to Discussion: 4,678, Manuscript: 8,295, Abstract: 250.
References: 60
Tables and Figures: 2/2.

Short title: neurofilaments in contact sports.

Keywords : Neurofilaments, physical contact sports, athletes, brain tissue damage, chronic traumatic encephalopathy.

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Disclosures

Carl Verduyn reports no disclosures.

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Johnny Duerinck reports no disclosures.

Sebastiaan Engelborghs reports no disclosures.

Koenraad Peers reports no disclosures.

Jan Versijpt reports no disclosures.

Miguel D'haeseleer reports no disclosures.

ABSTRACT

Objective

To evaluate whether participating in physical contact sports is associated with a release of neurofilaments and whether this release is related to future clinical neurological and/or psychiatric impairment.

Methods

We have performed a systematic literature review of the PubMed, MEDLINE and Cochrane Library databases using a combination of the search terms neurofilament(s)/intermediate filament and sport(s)/athletes. Only original studies, written in English, that have reported on neurofilament measurements in CSF and/or serum/plasma of physical contact sport athletes were retained for data extraction. This review was conducted following the Preferred Reporting Items for Systematic Review and Analyses guidelines.

Results

Eighteen studies reporting on light chain neurofilament (NfL), in eight different contact sports (i.e. boxing, American football, ice hockey, soccer, mixed martial arts, lacrosse, rugby and wrestling), matched our inclusion criteria. In 13/18 athlete cohorts, significant NfL elevations in CSF or serum/plasma were described, as compared to situations of non-exposure. Most compelling evidence was present in boxers and American football players, where marked and/or immediate exposure-related increases were appreciable at the intra-individual level in exclusive cohorts, generally paired with control groups who were well-descibed and matched for age. Findings in others sports were less suitable to draw conclusion upon. No studies were encountered that have investigated the relationship with the targeted clinical endpoints.

Conclusion

Nfl release can be seen, as a potential marker of neuronal brain damage, in participants of physical contact sports, particularly boxing and American football. The exact significance regarding the risk for future clinical impairment remains to be elucidated.

INTRODUCTION

Sports-related concussion (SRC) is a less severe form of traumatic brain injury (TBI) commonly occurring among physical contact sports practitioners, with approximately three million cases annually in the United States alone.[1] Diagnosis of concussion is established on clinical grounds but may be complicated due to its often vague, subtle and/or subjective presentation,[2] while conventional neuroimaging and electrophysiological studies only seldomly demonstrate clear abnormalities.[3, 4] Consequently, there is a need for reliable biomarkers to support SRC management, ranging from facilitating detection in pauci-symptomatic subjects to identifying those at risk for an unfavorable outcome, which is further stressed – albeit still a matter of debate – by several studies suggesting a harmful accumulative effect of repeated subclinical impacts on brain health.[5-8]

Neurofilaments are structural proteins expressed exclusively and abundantly in neurons, making them promising candidate biomarkers of neuronal damage. Current research has predominantly focused on light chain neurofilament (NfL), demonstrating increased levels – generally associated with present and future neurological disability – in the cerebrospinal fluid (CSF) and serum/plasma of patients with multiple sclerosis (MS), stroke, TBI, Alzheimer's disease and several other neurodegenerative disorders.[9] The emergence of NfL as a marker of neuronal damage may enable better diagnostics in individuals with suspected SRC and could offer new strategies to investigate the potential safety risks of collision sports. The purpose of this review was to systematically overview the literature on CSF and serum/plasma neurofilament assessments in physical contact sport athletes, and to gain insights in the clinical significance of elevated values within this context.

METHODOLOGY

Design and aims

The Patient/Problem/Population, Intervention, Comparator and Outcome (PICO) structure, which is a well-acknowledged approach in evidence-based medicine to frame and answer health-care related queries,[10] was used to translate our global purpose into a concrete research question. Through this systematic review, we wanted to study whether participating in physical contact sports (P) is associated with increased neurofilament levels (O_1) in CSF and/or serum/plasma samples (I), as compared with situations of non-exposure (i.e. in-person and/or group comparisons) (C), and, if so, whether such elevations are associated with future clinical neurological and/or psychiatric impairment (O_2). Structured working objectives

(WOs) were used to organize the results of the literature search, for reasons of comprehensiveness, as follows: (a) collecting all papers deemed relevant to address our research question while adding a standardized quality assessment, (b) grouping the retrieved literature according to whether or not reporting elevated neurofilament levels in the population of interest, (c) identifying information on the relationship between such increases and the targeted clinical outcome measures, (d) classifying the most relevant findings per type of sport, e.g. outcome measure not corresponding to O_2. This review was conducted using the recommendations of the Preferred Reporting Items for Systematic Review and Analyses as guidance.[11] The study was registered in the PROSPERO: Internal Prospective Register of Systematic Reviews database (CRD42020192712), maintained by the Centre for Reviews and Dissemination at the University of York (Heslington, UK), to help avoid duplicate efforts.

Search strategy

International peer-reviewed literature on contact sports-related neurofilament measurements up to June 30, 2020 was screened using the PubMed, MEDLINE and Cochrane Library databases. Medical Subject Heading search terms were entered in all fields of publication (e.g. abstract, title, keywords) and consisted of the following sequence: 'neurofilament OR neurofilaments OR intermediate filament OR intermediate filaments' AND 'sport OR sports OR athletes'. The abstracts of all retrieved papers were critically evaluated for relevance, with additional reading of the entire text in case of uncertainty. Studies (both with and without SRC context) were declared eligible for data collection and inclusion if they were (1) written in English, (2) reported data assessed in humans and (3) included contact sports-related measurements of neurofilaments in CSF and/or serum/plasma. Contact sports were defined as sports during which physical contact between participants is an inherent or almost inevitable part of the regular game (see Table 1 for a list of all encountered sports during the search). Reviews, case reports and case series were rejected; there were no other exclusion criteria. Bibliographies were reviewed in order to identify complementary studies (who subsequently underwent the same evaluation procedure) not found by the initial search. Two authors (CV and MD) independently performed the search and selection procedure. Disagreements were resolved through discussion and consensus between both reviewers.

Data collection

Full-text versions of all papers meeting the inclusion and exclusion criteria (n = 18) were obtained and read by the first and senior author(s) of this review. Data were extracted by CV

and MB using a standardized form that included the year of publication, first author, journal, global study aim, study design, number of subjects, type of contact sport, age of subjects, type of body fluid analysed, analytical method, sample time points and collection times, outcome measures, results, *P* value and main conclusions. An composite evidentiary Excel spreadsheet (Microsoft Corp, Redmond, WA, USA) compiling all variables was created to ensure reproducibility and completeness of the dataset. MD reviewed this file and any unclarities or disagreements on the inserted data were resolved through discussion between MD, CV and MB. As a priori expected, results were considered too heteretogenous (e.g. multiple sport types) to perform a meta-analysis.

Quality assessment

The methodological quality of each included paper was evaluated using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (National Institutes of Health, Bethesda, Ma, USA). The same rating scale was applied (yes = 1, no = 0, not mentioned = 0) for each of the 14 questions on this checklist and final study quality was determined by adding the individual scores. Consequently, papers were rated as good (total > 9), fair (4 < total < 10), or poor (total < 5).[12] This quality assessment was conducted independently by CV and MD. Variations were discussed until a consensus was reached.

RESULTS

WO/a: literature overview

Our search yielded eighteen original research papers suitable for data extraction. A detailed flowchart of the selection process is shown in Figure 1. All papers were published between 2006 and 2020. Most of them (16/18) were based on prospective data collection. We differentiated 15/18 longitudinal from 3/18 cross-sectional trial designs. NfL was the only neurofilament subtype for which data were available; eight papers reported on serum, five on plasma and five on CSF assessments. Sample sizes for the total cohorts (i.e. also including controls) varied from 11 to 504 subjects; yet, more than 60% of the studies recruited less than 50 subjects while statistical sub-analyses on even smaller samples were often conducted in these trials, making them vulnerable to effects driven by chance/outliers. Age ranged from 17 to 68 years and the majority of studies (9/13) involved a well-matched (with regard to age) and -defined (i.e. no known history of head trauma or neurological disease) control group, if present. Heterogeneity mainly concerned contact sport athletes characteristics (e.g. reported history of cumulative head trauma) affecting interpretability in studies with intra-individual

comparisons, variability in exposure modality and severity and pre-analytical factors such as timing of sampling in relation to exposure. Notably, the larger number of participants were men. Other demographical information, such as weight and body mass index (only reported in REF 20 and 25, respectively), was rather scarce. Core characteristics and findings of the individual studies are listed in an evidentiary overview; data available from Dryad (Supplementary table) at https://doi.org/10.5061/dryad.dz08kprw4.

One paper reported on serum NfL measurements in two separate cohorts (i.e. boxing and ice hockey),[13] while a single cohort of boxers was used to generate two different publications by one and the same Swedish research group.[14, 15] Thus, overall, eighteen unique cohorts of contact sport athletes were withheld, and our evidentiary overview was organized accordingly (cohorts were named as 'first author + year of first publication' - results of REF 14 and 15 were merged under cohort 'Neselius 2012', while REF 13 was split out as cohorts 'Shahim 2017a' and Shahim 2017b'). Findings will from here on be mentioned in the text with reference to these unique cohorts. Eight specific types of contact sports were represented (exclusive cohorts: boxing 3/18, American football 3/18, ice hockey 3/18, soccer 4/18 - mixed cohorts: American football plus ice hockey 1/18, boxing plus mixed martial arts plus retired boxers 2/18, American football plus ice hockey plus lacrosse plus rugby plus soccer plus wrestling 1/18, retired American football plus retired ice hockey plus retired boxing 1/18). It is expected that cohorts Bernick 2018 and 2020 (mixed fighters) have a substantial overlap since both were generated from the same database (see below).[16, 17] Key information derived from the cohorts, based on the content of the Appendix and supplemented with quality assessment scores for each individual study, is displayed in Table 2.

WO/b: elevated neurofilament levels?

Significantly elevated NfL levels in CSF or serum/plasma were found in 13/18 unique cohorts of contact sport athletes, as compared with controls and/or intra-individual situations of non-exposure (see indications in Table 2). Timing of sampling in relation to injury has previously been shown to be relevant in order to capture the peak NfL values which are expected to be situated around day 21,[18] and varied in our studies from one hour after exposure to 189 days with a relative dearth of sampling close to this assumed optimum (Figure 2). Notably, in four cohorts (including the three exclusive ice hockey cohorts) biochemical assessments associated with exposure were only performed within the context of a clinically apparent SRC. No NfL alterations were seen in the mixed cohorts (3/4) which contained non-fighters,[19-21] or when

exploring the impact of standardized heading sessions in two cohorts of soccer players.[22, 23] This latter finding, however, was contradicted by observations in two other cohorts.[24, 25]

WO/c: relationship with future disability?

There were no studies investigating the relationship between contact sport-associated NfL increases and the later occurrence of clinical neurological and or psychiatric impairment. Quantifiable clinical rating (e.g. neuropsychological evaluation and/or concussion severity) around the time of biochemical sampling was included in eight cohorts. Interestingly, associations between plasma NfL levels and brain volumetry on magnetic resonance imaging (MRI) were examined in three cohorts (i.e. the mixed cohorts which included fighters).[16, 17] More detailed findings are provided below per type of sport.

WO/d: most important findings per sport

Boxing

Significantly elevated levels of NfL in CSF or serum samples, obtained shortly after a bout (1-6 or 7-10 days), were observed in three different cohorts of amateur boxers, as compared with healthy non-boxing controls.[13, 14, 26] These control groups were age-matched and did not include subjects with a history of head trauma or neurological diseases. In one of the studies, it was also shown that athletic (gymnast) and non-athletic controls were inseparable with regard to their NfL levels; all narrowing down potential biological confounders. Values in boxers decreased following a 3-month rest period but did not reach control levels at that moment. The initial increase was more pronounced in boxers who had received many or high-impact hits to the head and NfL could be used with high accuracy (AUC = 0.97) to differentiate boxers from controls, as well as boxers exposed to high- versus low-impact, 7-10 days after bout.[13, 26] Neselius and co-workers found that neuropsychological test results (including memory, processing speed and executive function) were comparable between boxers after a bout and control subjects; yet, boxers with NfL levels that were still elevated in the CSF (defined as two standard deviations above the control mean) after at least 14 days of rest did demonstrate impairment on the Trail Making A and Simple Reaction Time test suggestive of executive dysfunction.[15]

The Professional Fighters Brain Health Study (PFBHS) is a longitudinal observational project, initiated by the Cleveland Clinic in 2011, annually collecting blood samples, brain MRI and cognitive testing in active and retired boxers, active mixed martial arts fighters and

control subjects (controls were age-matched with the active fighters and neither had a history of head trauma or neurological disorders). Plasma NfL levels from 471 participants significantly differed across the four groups, with the highest levels observed in the active boxers. Higher values at baseline cross-sectionally correlated with lower regional brain volumes (thalamus, hippocampus and corpus callosum) and worse cognitive performance (psychomotor and processing speed) in the active fighters.[16] In another cohort derived from the PFBHS, active fighters had a more rapid yearly decline, as compared with control subjects, in thalamic and anterior corpus callosum volume, of which the latter was associated with higher baseline NfL values.[17]

Mixed martial arts

A pattern of accelerated thalamic and callosal volume loss, as compared to controls, was also noted in the mixed martial arts fighters of the Bernick 2020 cohort, in line with what was seen in the active boxers.[17] However, In the Bernick 2018 cohort, mean NfL values in active mixed martial arts fighters (14.58 ± 0.86 pg/mL) were only tending towards an increase, as compared with controls (11.27 ± 1.40 pg/mL).[16]

American football

American football has one of the highest incidences of SRC among all team sports. Serial assessments in two separate cohorts demonstrated progressively increasing serum NfL levels over the course of a playing season (in parallel with an augmenting likelihood of accumulating head impacts) in collegiate American football players, particularly in those categorized as first-team starters. NfL levels were already significantly different between groups (control swimmers < non-starters < starters) at baseline measurement, indicating an effect of past exposure that had not normalized after a 9-week rest period.[27, 28] Even during summer training camp, pre- to post-practice increases in plasma levels of NfL were associated with the frequency and magnitude of head impacts, measured using an accelerometer-embedded mouth protection.[29] On the contrary, one study consisting of a mixed cohort (n = 11) of American football or ice hockey players failed to show any significant difference between serum NfL levels measured in the pre-season and those obtained at day 6 and 14 after suffering a SRC.[19] Similar observations were made in a larger, more heterogeneous, cohort of American collegiate athletes. But when these athletes were further subdivided according to severity of the SRC, based on the presence of post-traumatic amnesia of loss of consciousness, mounting levels of NfL were seen over the post-injury time points with

significantly increased values at the day of return to play (median of 7 days after exposure) and 7 days thereafter.[20] Taghdiri and co-workers did not find differences in serum NfL levels between healthy controls free from previous head trauma and a mixed cohort of retired contact sport athletes, with a history of SRC, of which the majority were former American football players. However, NfL levels in the group of ex-athletes positively correlated with baseline mean diffusivity (i.e. a diffusion tensor MRI parameter likely reflecting disruption of white matter integrity), as well as with the amount of its increase over 2 years of follow-up, in the corpus callosum.[21]

Ice hockey

Mono-disciplinary studies in professional ice hockey only involved athletes who suffered a clinically apparent concussion during play. Serum NfL concentrations were elevated, as compared with controls not known to have a neurological condition or history of head trauma, and increased over time, i.e. measurements performed from one to 144 hours after the concussion, in two cohorts. Interestingly, higher values were associated with a longer duration of post-concussion symptoms and a delayed return to play.[13, 30] Corresponding observations were made in a study based on CSF analysis.[31]

Soccer

Head collision with opponents and/or teammates is less inherent to the game of soccer (compared to e.g. American football and ice hockey) but players may be exposed to repeated low-severity TBI due to heading. This hypothesis could not be supported by a Swedish study published in 2007, in which no significant differences in CSF NfL levels were seen between age-matched cohorts of soccer players and healthy non-athletic controls, who both performed a training session of standardized headings (one group of 10 headings and one of 20). Samples were obtained 7-10 days after exposure.[22] In contrast, two other cohorts did demonstrate elevated plasma NfL levels when measured more early after a similar heading challenge (one and 24 hours, respectively), as compared with baseline levels.[24, 25] The most pronounced NfL change (26% at one hour and 311% at 22 days) was found in the cohort wherein the highest number of headings were performed and in which the presence of immediate TBI was further supported by elevations in total number and severity of concussion-related symptoms, assessed one hour after impact with the Standardized Concussion Assessment Tool-3rd edition.[24] In the second positive study, there was an increase in NfL at 24 hours post-exposure but the magnitude was very modest (1.2 times)

comparably.[25] Notably, all NfL values reported in this trial (in controls and athletes) were less than half of the control values reported in all other blood-based studies, using the same technology, coming from our search. A very recent and considerably larger study by Sandmo and co-workers evaluated the effect of high-intensity physical exercise (no heading), a repetitive heading training session (mean of 19 headings) and head impacts during a match (accidental SRC), respectively, in a cohort of Norwegian professional soccer players, but found no differences in serum NfL measured one hour after exposure, as compared to pre-seasonal baseline values. In addition, no effects on baseline NfL levels were seen in players with a self-reported high-risk (≥ 1 previous concussion and ≥ 11 headers per match) versus low-risk (no previous concussion and 0-5 headers per match) profile at pre-season,[23] in contrast to findings pointing towards a cumulative effect in American football.

Lacrosse, rugby and wrestling

Lacrosse, rugby and wrestling were only represented as minority contributors (less than 20% each, 28% combined) to one mixed cohort.[20] No specific conclusions regarding these activities could be drawn from the available literature.

DISCUSSION

SRC is a major public health issue but its true incidence may still be underestimated as routine clinical, electrophysiological and radiological evaluations seem to lack sensitivity and specificity to properly map the effects of traumatic stress to the brain. Safety concerns regarding repetitive and potentially sub-concussive head impacts occurring during play in physical contact sports, particularly boxing and American football, have intensively been discussed in the lay media and scientific community over the past decade. In parallel, neurofilaments in CSF and/or serum/plasma have developed as a promising non-invasive and quantifiable marker of neuro-axonal damage in various neurological disorders, including TBI. Such advances in biochemical research may prove to be paramount in the future progress of this field. To the best of our knowledge, the present article is the first which systematically overviews the available literature on neurofilament measurements in physical contact sport athletes. Our search identified only eighteen papers, spanning eight different disciplines, suitable for data extraction, demonstrating that the domain of interest is still in its infancy. Quality was rated as good in 6/18 and as fair in 12/18 of the studies, but NfL was the only neurofilament subtype for which results were published.

When addressing the first arm of our research question, the majority of studies (i.e. 13/18 unique cohorts) argued for a positive association between the contact sport of interest and NfL released by damaged neurons. We decided to give a sport-by-sport listing for reasons of comprehensiveness and specificity, and, as expected, most compelling evidence was present in boxers and American football players, where immediate exposure-associated increases were appreciable at the intra-individual level in exclusive cohorts.[13, 26-29] The accuracy of NfL to discriminate boxers from controls after a bout was high, even separating out those with low exposure, with levels remaining elevated following a subsequent rest period of 3 months. Negative findings in American football originated from mixed cohorts and could partly be explained by differences in exposure severity in combination with suboptimal sampling time-points in relation to impact. Furthermore, the cumulative effect in players may complicate the interpretability of intra-individual results, possibly even obscuring otherwise positive findings. Cohorts with mixed martial arts fighters, lacrosse athletes, rugby players or wrestlers always included practitioners of other sports as well, limiting the ability to draw specific conclusions. Findings in ice hockey should be interpreted with caution since all respective exclusive cohorts were examined in the context of a clinically apparent SRC. Increased neurofilament levels have also been reported in blood compartments of subjects affected by severe and mild TBI regardless of the cause,[32, 33] including in athletes who were not necessarily involved in a contact sport.[34, 35] Head collisions are probably more accidental than inherent to the normal course of ice hockey, and the reported elevations may thus be more informative on the concussion/brain trauma in se than on the nature of the game.

Effects of standardized headings were conflicting in soccer players, which could not be explained by marked variation in age, comparator, examined body fluid, timing of sampling or analytical method. The number of participants was substantially larger in the Sandmo 2020 cohort in which, compared to baseline, no relevant change in serum NfL was seen one hour after a training session of approximately twenty standardized headers, whereas magnitude of exposure favors the positive Wallace 2018 study. Timing of the sampling procedure, with regard to impact, might be also particularly worth highlighting here since Figure 2 suggests that, in general, NfL levels of contact-sport athletes begin to increase within the first week after exposure, show their highest point between day 7 to 22 and start to return to normal at month 3. This pattern corresponds to findings in acute stroke where peak NfL values were observed 3 weeks after injury,[18] and indicate that the optimal sampling window might be close to 21 days after exposure, of course likely also depending on the exposure. The Wallace

2018 study best respected this reasoning but larger studies including well-defined, age-matched, soccer player and control cohorts that follow the same standardized operating pre-analytical and analytical procedures are needed to settle the above discrepancies. Safety of intentional heading in soccer remains a matter of debate,[36-38] although some preliminary clinical (i.e. having a higher number of headings was associated with an augmented burden of neurological symptoms, both assessed with questionnaires) and radiological (self-reported number of headings was associated with abnormal white matter microstructure on diffusion tensor MRI) findings also defend the theory that such challenges can have a traumatic effect on the brain.[39, 40]

Data reflecting the clinical relevance of NfL elevations in contact sport athletes are still scarce and the results of our search did not allow to address the second part of the research question. No studies were found which have explored the effect on long-term neurological and/or psychiatric endpoints. Some protocols did incorporate clinical assessments at the time of biochemical sampling, including cognition in active fighters,[15-17] but we have left this out of scope of our query as we felt that potential point-associations could also be largely driven by acute concussive events. Nonetheless, the encountered relationship between increased NfL and worse (short-term) symptomatic outcome in ice hockey players suffering from a SRC advocates for the potential of this biomarker as clinical prognostic tool. Chronic traumatic encephalopathy (CTE) is a heterogeneous neurodegenerative syndrome associated with TBI but which is still awaiting full clinical and pathological characterization, not to mention validation. Medical history and risk profile may provide suggestive clues but the diagnosis can only be confirmed after post-mortem brain tissue examination.[41, 42] No studies exploiting the role of neurofilament levels assessed during life in subjects affected by CTE have been published so far. In the field of MS, a frequently occurring autoimmune demyelinating and degenerative disorder of the central nervous system,[43] it has been shown that elevated NfL levels in serum and/or CSF are able to predict accelerated brain volume loss over periods up to 15 years.[44, 45] Brain atrophy in individuals with MS, as compared to healthy age- and sex-matched controls, is associated with both present and upcoming cognitive dysfunction, while more recent work has also established the predictive value of increased serum NfL values directly on decline of cognition.[4(1)6] Similar findings have been reported across the more classic dementia spectrum suggesting a proof-of-concept for degenerative brain pathologies.[9, 47-50] Contact sport athletes appear to be particularly vulnerable to volume loss of the thalamus, corpus callosum, hippocampus and amygdala.[17, 51] Higher baseline plasma NfL in

the active fighters of the Bernick 2020 cohort were associated with a greater atrophy rate in some of those regions,[17] and, in the Taghdiri 2020 cohort of retired professional contact sport athletes, serum NfL positively correlated with the increase of other, more advanced, MRI metrics of neurodegeneration in the corpus callosum over the course of two years.[21] Strikingly, a series of studies have pointed out that the neuropathological changes of CTE/repetitive TBI may also appear in individuals long after surviving a single episode of brain trauma,[42] but it is also worth noting that in a large study conducted in the Canadian province of Manitoba, the neuropathological changes of CTE were found in a substantial proportion of brains of deceased individuals who had no history of trauma, particularly among those known with substance abuse.[52]

This review is not without limitations. First, the number of studies corresponding to our search criteria was limited and they suffered from rather small sample sizes. Sex distribution was heavily male-orientated and studies in children, where head impacts could have even more detrimental effects, are non-existing. Notably, a very recent publication described significant alterations of structural and functional connectivity in the brain, as compared to non-contact sport athletes, of 73 non-concussed female rugby players.[53] Second, neurofilament research is not free from possible biological confounders. These proteins are specific for neurons but a release can occur on a variety of causal backgrounds, such as trauma, inflammation, ischemia and primary degeneration. Blood and CSF levels tend to increase with growing age,[54, 55] and there is a general lack of understanding how other comorbidities can affect the measurements. For instance, blood NfL levels have recently been found to correlate with body mass index and blood volume.[56] Elevations in serum/plasma can also originate from pathologies affecting the peripheral nervous system,[57, 58] which may introduce bias in physical contact sport athletes as they are susceptible to injury of body parts other than the head. Third, NfL levels are informative at a group level but cut-offs for distinguishing abnormal from physiological values in individual subjects have not yet been defined, which tempers their usefulness in current clinical practice. One of the most pertinent issues in sports medicine concerns return to play decisions in concussed athletes, which cannot be guided by neurofilaments as long as this problem remains unsolved. Fourth and finally, this review has not taken into account other biochemical markers of TBI which are currently under investigation in athletes, such as tau.[19, 59] Cortical neurofibrillary tangles of hyperphosphorylated tau have been recognized as an important neuropathological finding in CTE,[41, 42] which has recently been linked with matching positron emission tomography

changes and plasma NfL elevations in living war veterans.[60] Yet, it must be emphasized that CTE likely is not an exclusive tauopathy,[41, 42] and CSF levels of tau correlate less well to those in blood than what is the case for NfL.[16]

In conclusion, CSF and serum/plasma levels of NfL appear to be increased, as a newly emerging biomarker of neuro-axonal damage, in participants of physical contact sports, particularly boxing and American football. The clinical significance of such elevations remains unclear but findings from the PFBHS suggest an association with accelerated brain atrophy, which may, in turn, be considered as a risk factor for cognitive decline. We advocate for future studies to be discipline-specific and remain as close to the regular sport practice as possible. Longitudinal and multimodal research designs seem warranted to properly understand the significance of raised NfL outside the initial potentially concussive event. CTE entails too many unclarities of its own and cannot be reliably diagnosed in a timely manner to serve as a trial endpoint at this moment. Behavior and cognition are distributed diffusely in the brain and appear to rely on dynamic interactions between distinct regions operating in higher network structures, which can be assessed by advanced MRI methods such as functional and diffusion tensor imaging. Demonstrating early disruptions in such networks could, for example, be very informative and illustrate one possible step in the advised way forward.

AUTHOR CONTRIBUTIONS

CV and MD conceptualized the project. CV wrote the first the first draft of the manuscript. All authors, with a more substantial role for MD and MB, were involved in the critical reviewing.

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FIGURE LEGENDS

Figure 1: PRISMA flow diagram of the selection process.

PRISMA = Preferred Reporting Items for Systematic Review and Analyses, Nf = neurofilament.

Figure 2: Increase light chain neurofilament in contact sport athletes in relation to time from injury/exposure.

NfL = light chain neurofilament.

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<u>Appendix 1</u> - Authors

Included	**Excluded**
• American football • Boxing • Ice Hockey • Lacrosse • Mixed martial arts • Rugby • Soccer • Wrestling	• Diving • Swimming • Tennis

Table 1: List of encountered sports during the literature search

Cohort[A]	**Subjects *n* (age)[B]**	**Samples**	**Timing of sampling[C]**	**Controls *n* (age)[B]**	**Main study outcomes with regard to NfL levels**	**Study quality**
Boxing						
Zetterberg 2006*^ REF 26 – P/L	1) 14 (22) 2) 13 (NA)	CSF	1) 7-10 d 2) 3 m	10 (30) healthy ++ non-athletes	Increased after bout (6.8 times) and after 3 m of rest (1.7 times), versus controls. Increased after bout (4.1 times), versus after 3 months of rest. Increased after bout (7.5 times) in boxers receiving many (n = 7; hits > 15) versus few (n = 7; hits < 1) hits to the head.	9/14
Neselius 2012* REF 14/15 – P/L	1) 30 (22) 2) 26 (24)	CSF	1) 1-6 d 2) 14 d	25 (22) healthy + non-athletes	Increased after bout (3.9 times) and after 14 d of rest (3.0 times), versus controls.	9 and 7/14[e]
Shahim 2017a* REF 13 – P/L	1) 14 (22) 2) 13 (NA)	Serum	1) 7-10 d 2) 3 m	14 (24) healthy ++ non-athletes and 12 (19) healthy ++ gymnasts	Increased after bout (5.7 times) and after 3 m of rest (2.1 times), versus controls. Increased after bout (3.3 times) in boxers receiving many (n = 7; hits > 15) versus few (n = 7; hits < 15) hits to the head. High accuracy (AUC = 0.97) after bout in discriminating between boxers and controls, and between boxers receiving many versus few hits. No difference between athletic and non-athletic controls.	9/14

American football						
Oliver 2016* REF 27 – P/L	11 (20) starters and 9 (20) non-starters (20)	Serum Serum	9 w (T_1), FU 189 d (T_{2-8})	19 (20) swimming athletes	Increased after 9 w of rest (1.2 and 1.3 times, respectively) in starters, versus non-starters and controls. Progressive increase over playing season in starters (T_8: 2.0 and 2.2 times, respectively), versus baseline and non-starters.	10/14
Oliver 2018* REF 28 – P/L	20 (21) starters and 15 (20) non-starters	Plasma	14 w (T_1), FU 120 d (T_{2-7})	NA	Increasing throughout playing season (1.5 times versus non-starters), versus baseline and non-starters. Discriminating between starters and non-starters during the playing season (T_{2-3}, T_{5-7}; AUC 0.69-0.76).	10/14
Rubin 2019* REF 29 – P/L	12 (21) with high-impact and 6 (21) with low-impact		2 m pre-practice, 1 h pre- and post-practice	NA	Positive correlation (rs = 0.49-0.54) between pre- to post-training increase and head impacts during training session. No significant difference in absolute values between high- and low-impact groups.	11/14

Ice hockey						
Shahim 2016*^ REF 31 – P/CS	16 (31) with post-SRC symptoms > 3 m	CSF	*Mdn* 4 m	15 (25) healthy ++ unspecified	Increased (2.0 and 1.7 times, respectively) in players with symptoms persisting for > 1 y, versus players whose symptoms resolved in 1 y and controls. Correlating with lifetime concussion event in players (rs = 0.52).	10/14
Shahim 2017b* REF 13 – P/L	28 (27) with SRC	Serum	1, 12, 36 and 144 h	14 (24) healthy ++ non-athletes	Increased (1.3-1.4 times) in players with symptoms persisting > 6 d, versus controls, from 1-144 h; diagnostic accuracy (AUC) ranged from 0.79-0.74.	9/14
Shahim 2018* REF 30 – P/L	87 (26) with SRC	Serum	1, 12, 36 and 144 h	19 (25) healthy ++ non-athletes	Increased (1.2 times) in players, versus pre-season baseline and controls. Increased (1.2-1.5 times) in players with symptoms persisting > 10 d, versus players whose symptoms resolved in < 10 d, over the period of 1-144 h; diagnostic accuracy (AUC) ranged from 0.72-0.82.	10/14

Soccer						
Zetterberg 2007 REF 22 – P/CS	13 (23) with 20 headings and 10 (26) with 10 headings	CSF	7-10 d	9 (24) healthy + non-athletes	Unaltered in players performing 10 or 20 headings, versus controls.	9/14
Wallace 2018/1* REF 24 – P/L	11 (24)	Serum	1 h and 22 d	NA	Increased at 1 h (26%) and 22 d (n = 7; 311%) after performing 40 headers, versus baseline.	9/14
Bevilacqua 2019* REF 25 – P/L	18 (20)	Plasma	0, 2 and 24 h	16 (21) kicking players	Increased levels (1.2 times) at 24 h after performing 10 headers, versus baseline and controls.	11/14
Sandmo 2020^ REF 23 – P/CS	[1)] 47 (NA) with high-intensity exercise, 47 (NA) with headings and 35 (NA) with SRC	Serum	1 and 12 h	[1)] NA [2)] 57 (NA) low-risk players: no previous SRC	[1)] No short-term effects (1 to 12 h) after one training session with high-intensity exercise (no heading), repetitive heading (mean 19 times) or after a match with accidental SRC, versus pre-season baseline. [2)] No long-term/cumulative effects at pre-season baseline in players with a high-risk profile of previous head-impact exposure, versus players with a low-risk exposure profile.	9/14

	[2)] 23 high-risk players: ≥1 previous SRC & ≥11 headings/ match					

Mixed						
Wallace 2018/2[a] REF 19 – P/L	11 (NA) with SRC	Serum	6 and 14 d	NA	Unaltered at d 6 and 14 following a SRC, versus pre-season baseline.	8/14
Bernick 2018[b]* REF 16 – R/L	52 (48) rB, 117 (30) aB, and 169 (30) aMMA	Plasma	rB: > 2 y; aF: > 45 d and < 2 y	79 (31) healthy ++ unspecified	Increased (1.9 and 1.5 times, respectively) in aB versus controls and aMMA. Higher baseline levels associated with lower baseline volumes of thalamus, hippocampus and central/posterior corpus callosum on MRI ($p = 0.005$-0.047) and lower performance in psychomotor and processing speed ($p = 0.02$ and 0.04, respectively).	8/14
Bernick 2020[b]* REF 17 – R/L	23 (46) rB, 50 (29) aB and 100 (29) aMMA	Plasma	rB: > 2 y; aF: > 45 d and < 2 y	31 (31) healthy ++ unspecified	Higher baseline levels were associated with decline in hippocampus ($p < 0.001$) and midanterior corpus callosum ($p = 0.015$).	6/14
McCrea 2020[c] REF 20 – P/L	264 (19) with SRC	Serum	Acute post-injury, 24-48 h, at PRA and 7 d after RTP	138 (19) contact sport athletes and 102 (19) non-contact sport athletes	Unaltered at all time points in athletes with SRC, versus controls and pre-seasonal baseline. Increased over post-injury time-points in athletes with SRC who had post-traumatic amnesia or loss of consciousness (n = 57), versus those without and controls.	8/14

Taghdiri 2020[d] REF 21 – P/L	52 (55) ExPros with SRC history	Serum	NA	21 (49) healthy ++ unspecified	Unaltered at baseline in ExPros, versus controls. Positively correlating with MD of fornix and corpus callosum, with increased MD (> 2 years) in corpus callosum and with total ventricular volume. Negatively correlating with memory and speed of processing scores.	8/14

[A] Cohorts are named as 'first authors + year of publication', matching reference – study design can be found under each cohort name.

[B] Age is reported as mean or median, details can be found in the Supplementary table (see Dryad at https://doi.org/10.5061/dryad.dz08kprw4).

[C] With regard to the moment of exposure.

[a] American football plus ice hockey, [b] boxers plus mixed martial arts plus retired boxers, [c] American football plus ice hockey plus lacrosse plus rugby plus soccer plus wrestling, [d] retired American football plus retired ice hockey plus retired boxing, [e] 9/14 applies to REF 14 and 7/14 applies to REF 15.

Cohorts in which the contact sport of interest was associated with elevated NfL levels are indicated by *.

Healthy + = no known history of a neurological disorders, healthy ++ = no known history of a neurological disorders or head trauma.

^ Matching and/or statistical correction for age between contact sports athletes and controls not mentioned.

n = number, NfL = light chain neurofilament, REF = reference, P = prospective, L = longitudinal, NA = not available, CSF = cerebrospinal fluid, d = day(s), m = month(s), AUC = area under the curve, w = weeks, FU = follow-up, h = hour(s), CS = cross-sectional, SRC = sports-related concussion, *Mdn* = median, R = retrospective, rB = retired boxers, aB = active boxers, aMMA = active MMA fighters, aF = active fighters, MRI = magnetic resonance imaging, PRA = point of reporting being asymptomatic, RTP = return to play, ExPros = ex-professionals, MD = mean diffusivity.

Table 2: Key information obtained from the available unique cohorts of contact sport athletes.

1. Jakimovski D, Kuhle J, Ramanathan M, Barro C, Tomic D, Hagemeier J, et al. Serum neurofilament light chain levels associations with gray matter pathology: a 5-year longitudinal study. Ann Clin Transl Neurol. 2019;6(9):1757-70.