

# Predictive performance of blood S100B in the management of patients over 65 years old with mild traumatic brain injury

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 with mild traumatic brain injury

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#### 1 ABSTRACT

Background: We previously assessed the inclusion of S100B blood determination into clinical decision rules for mild traumatic brain injury (mTBI) management in the Emergency Department (ED) of Clermont-Ferrand Hospital. At the 0.10 µg/L threshold, S100B reduced the use of cranial computed tomography (CCT) scan in adults by at least 30% with a ~100% sensitivity. Older patients had higher serum S100B values, resulting in lower specificity (18.7%) and decreased CCT reduction. We conducted this study to confirm the age effect on S100B concentrations, and to propose new decisional thresholds for older patients.

9 Methods: A total of 1172 mTBI patients aged 65 and over were included. They were divided
10 into three age-groups: 65-79, 80-89, and ≥ 90 years old. S100B's performance to identify
11 intracranial lesions (sensitivity (SE) and specificity (SP)) was assessed using the routine 0.10
12 µg/L threshold and also other more efficient thresholds established for each age group.

13 **Results:** S100B concentration medians were 0.18  $\mu$ g/L, 0.26  $\mu$ g/L, and 0.32  $\mu$ g/L for the 65-14 79, 80-89, and  $\geq$  90 years old age-groups, respectively (p < 0.001). The most efficient thresholds 15 were 0.11  $\mu$ g/L for the 65-79 age-group and 0.15  $\mu$ g/L for the other groups. At these new 16 thresholds, SP was respectively 28.4%, 34.3%, and 20.5% for each age-group vs. 24.9%, 17 18.2%, and 10.5% at the 0.10  $\mu$ g/L threshold.

Conclusions: Adjustment of the S100B threshold is necessary in older patients' management.
An increased threshold of 0.15 µg/L is particularly interesting for patients ≥ 80 years old,
allowing a significant increase of CCT scan reduction (29.3%).

22 Keywords: biomarker, aging, dementia

#### 1 **INTRODUCTION**

Traumatic brain injury (TBI) constitutes a leading public health problem among older adults (> 60 years old) with an incidence of 275 per 100.000 in France (1). This is all the more important given the fact that the worldwide proportion of adults over 65 years old is expected to increase from 12.5% in 2010 to 20.0% by 2050, reaching this proportion by 2030 in industrialized countries (2).

7 Although the spectrum of TBI severity is variable, 80% of treated TBIs are mild (3). Mild traumatic brain injury (mTBI), is defined by a Glasgow Coma Scale (GCS) of 13–15 (4), and 8 according to clinical and anamnestic criteria, mTBI can be categorized into three prognostic 9 10 subgroups: mTBI with a low, medium or high risk of complications (5,6). The medium risk subgroup is particularly important as mTBI management guidelines recommend the use of 11 cranial computed tomography (CCT) for this subgroup of patients although the vast majority 12 (85%–99%) of the CCTs performed don't find significant intracranial lesions (7). In this 13 context, blood biomarkers can be used as a diagnostic tool for their ability to exclude 14 15 intracranial lesions thus reducing the use of CCT scans.

S100B protein, is a well-established TBI biomarker. It is a small dimeric protein (molecular 16 weight of 21 kDa) that consists of  $\beta\beta$  or  $\alpha\beta$  chains. S100B is commonly expressed in astrocytes 17 18 and Schwann cells but also in melanocytes, chondrocytes, adipocytes and skeletal muscle (8). S100B is involved in a variety of intracellular and extracellular regulatory activities in central 19 nervous system cells where it is highly expressed (9,10). Increased blood S100B levels are 20 21 associated with head trauma, in which damaged glial cells release S100B, it is then diffused into the bloodstream after passing through the blood-brain barrier (BBB). S100B is 22 subsequently eliminated by the kidneys, with a short half-life of 30-100 minutes (11). 23

The usefulness of S100B in the management of mTBI has been established in many observational research and confirmed in two interventional studies (12,13). The addition of

blood S100B measurement to the Scandinavian guidelines decreased significantly the need for 1 CCT scans. It allowed a one-third reduction of the CCT scans performed in adult mTBI patients 2 (14). In a previous study, we validated the inclusion of S100B into our clinical decision rules 3 for mTBI management in a cohort of 1449 patients presenting to the Emergency Department 4 (ED) of Clermont-Ferrand's Hospital (15). In this study, at a threshold of 0.10 µg/L, S100B 5 reduced the use of CCT scans by 33.4% with a sensitivity of approximately 100%. However, 6 older patients (> 65 years old), representing more than a third of the cohort, had higher serum 7 8 S100B values, resulting in a lower specificity of only 18.7% (15).

9 We conducted this study to evaluate in a larger cohort the effect of age on S100B 10 concentrations. The main outcome of this study is to propose new decisional thresholds ( $\neq 0.10$ 11 µg/L) adapted to older populations in order to improve S100B's specificity.

#### 1 <u>METHOD</u>

#### 2 Study design and patients

This study was carried out from June 2014 to March 2020 at the ED of Clermont-Ferrand 3 Hospital after approval by CPP Sud Est VI and in compliance with the Declaration of Helsinki 4 ethical principles regarding medical research involving human study participants. Patients were 5 informed of their right to express their disagreement regarding the use of their clinical 6 7 information for research purposes. Each patient referred to the ED for an mTBI (with a GCS  $\geq$ 13) was classified into one of three prognostic subgroups according to the SFMU's 8 recommendations (Société Française de Médecine d'Urgence) (5). The criteria regarding 9 10 patients' categorization in the three subgroups and the clinical decision algorithm are presented in Figure 1. 11

The inclusion criteria were as follows: an age  $\geq 65$  years old, an mTBI with medium risk of complications (*i.e.* with a GCS equal to 15 and antiplatelet treatment or loss of consciousness or retrograde amnesia 30 minutes before injury) and a time interval between injury and blood sampling being less than 3 hours (16).

The exclusion criteria were as follows: an age < 65 years old, an mTBI with a low risk of complications (*i.e.* asymptomatic patient without medium- or high-risk criteria and a GCS of 15), an mTBI with a high risk of complications (Figure 1) and a time interval between injury and blood sampling being greater than 3 hours.

Concerning the clinical decision consecutive to S100B measurements: patients with S100B levels below the well-established decision threshold of  $0.10 \ \mu g/L$  were categorized as having a low risk of complications and discharged (15). A CCT was strongly recommended within 8 hours for patients with S100B levels above the decision threshold (Figure 1).

For each patient who left the ED without a CCT, we checked if an hospitalization had occurredin the month following the mTBI.

#### **1** Serum S100B measurement

Serum S100B concentrations were determined by an electrochemiluminescence immunoassay using a Roche Diagnostics Cobas e411<sup>®</sup> instrument (Meylan, France). S100B results were available within 1 hour after sampling. Based on other studies in adults, the cutoff was set at 0.10 µg/L (17–19). Patients exhibiting serum concentrations below this threshold were considered S100B negative (S100B-), and those with concentrations above this cutoff were considered S100B positive (S100B+).

According to the manufacturer's instructions, the test system requires 18 min and a probe volume of at least 120- $\mu$ L serum. The lower limit of quantification (LLOQ) and lower limit of detection (LLOD) are 0.02  $\mu$ g/L and 0.005  $\mu$ g/L. Concentrations of up to 39  $\mu$ g/L can be measured without dilution. The intra-assay and inter-assay coefficients of variation (CVs) are <2.3% and < 5.6 %. The measurement uncertainty (calculated in the Biochemistry Department in Clermont-Ferrand, France) is 6% at a concentration of 0.183  $\mu$ g/L. This uncertainty must be taken into account when interpreting values close to the decision threshold (0.10  $\mu$ g/L).

#### 15 **Definition of Dementia**

In our study, patients suffering from dementia had one of the following neurological disorders:
Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia and
dementia of unknown etiology.

#### **19 CCT examination**

Each CCT scan was performed using a GE Healthcare Revolution GSI<sup>®</sup> according to the following protocol: a helical mode with a slice thickness of 2.25 mm, interval of 1.25 mm, 120 kV and a maximum of 280 mA from C1 to the top of the head with additional bone window reconstructions. To determine whether a patient had a trauma-relevant intracerebral lesion, the radiological parameters were recorded, and the patients were divided into two groups: one group with normal CCT scan for mTBI patients with no sign of trauma-relevant intracerebral lesion and another group with abnormal CCT scan for mTBI patients with at least one
 pathophysiological trauma-relevant intracerebral lesion (such as petechiae, meningeal or
 intraparenchymal effusion, pneumocephaly reflecting an osteomeningeal breach, compression
 or ventricular hemorrhage).

#### 1 Statistics

2 All statistical analyses were performed using Stata software (Version 15, StataCorp, College Station, US) with a two-sided type I error at 5%. Continuous data were presented as the median 3 and minimum (min), maximum (max) and interquartile range (IQR). To study the relationship 4 between S100B concentration and age, patients (n = 1172) were first, categorized into the 5 following age-groups: 65-69, 70-74, 75-79, 80-84, 85-89, 90-94,  $\geq 95$  years old. Then, they 6 were divided into three age-groups according to statistical distribution and to two by two 7 comparisons: 65-79, 80-89, and  $\geq$  90 years old. The comparisons between age-groups 8 concerning continuous parameters such as S100B concentrations were performed using 9 10 ANOVA or Kruskal-Wallis tests. The assumption of normality was assessed by the Shapiro-Wilk test and the homoscedasticity was evaluated using Bartlett's test. When appropriate 11 (omnibus p-value less than 0.05), post-hoc tests for multiple comparisons were applied: Tukey-12 13 Kramer after ANOVA and Dunn after Kruskal-Wallis. Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) were estimated using the 14 15 adult routine threshold of 0.10 µg/L for all patients and for each age-group. Then, based on the receiver operating characteristic (ROC) curve, the more efficient threshold (allowing the best 16 SP keeping a 100% SE) was evaluated for each age-group. For the 65-79 age-group, it was 0.11 17  $\mu$ g/L against 0.15  $\mu$ g/L for the two other age-groups. The comparisons for paired proportions 18 were performed using McNemar test whereas comparisons between groups for categorical data 19 were carried out using Chi-squared or Fisher's exact tests. Multivariable analysis (linear for 20 S100B as continuous variable and logistic for S100B according to threshold of 0.10ug/L and 21 optimal threshold) was conducted to take into account gender as confounder covariate. The 22 residuals from these models was analyzed as aforementioned. When appropriate, a logarithmic 23 transformation of dependent variable was applied. Sensitivity analyses were performed to 24

- 1 ensure the robustness of the results and conclusions, performing aforementioned analyses on
- 2 patients without intracranial lesions (n = 1109) and patients without dementia (n = 893).

#### 1 <u>RESULTS</u>

#### 2 Patient management and serum S100B concentration levels

3 Between June 2014 and March 2020, serum S100B assays were performed for 1314 patients. A total of 1172 mTBI patients were enrolled in this study, 142 patients, meeting the exclusion 4 criteria, were excluded (Figure 2). Of the 1172 patients, 504 were already included in our 5 previous study (15). The median interval between brain injury and blood sampling was 1.6 6 hours (min: 0.2 hours, max: 3.0 hours, IQR: 1.3-2.1 hours). The median serum S100B level was 7 0.23 µg/L (min: 0.03 µg/L; max: 8.97 µg/L; IQR: 0.12-0.46 µg/L). Of the 1172 patients 8 included in the study, 954 (81.4%) were S100B+ and 218 (18.6%) were S100B- (Figure 2). 9 10 Nevertheless, a CCT scan was performed for 67 S100B- patients, no abnormal scan was observed for these patients. Of the 954 S100B+ patients, 60 were discharged without CCT scan, 11 831 had a normal CCT scan and 63 had an abnormal CCT scan (Figure 2). For 127 patients 12 (10.8%), the decision algorithm (Figure 2) was not followed by the physicians (CCT scan 13 prescription despite S100B- or no prescription of CCT scan despite S100B+) (Figure 2). 14 15 Patients who left the ED without CCT scan did not develop any clinical signs suggestive of an intracerebral lesion in the month following mTBI. Moreover, we didn't find any hospitalization 16 nor any death for these patients. Therefore, patients without CCT scans were considered as 17 18 having no intracerebral lesions.

#### 19 **S100B levels according to age** (Table 1)

For patients aged 65 to 80, the median S100B concentration was 0.184 µg/L (min: 0.029 µg/L; max: 7.74 µg/L; IQR: 0.103-0.360 µg/L). For patients aged 80 to 90, the median S100B concentration was 0.255 µg/L (min: 0.032 µg/L; max: 8.97 µg/L; IQR: 0.129-0.474 µg/L). For patients aged 90 years and over, the median S100B concentration was 0.318 µg/L (min: 0.039 µg/L; max: 4.68 µg/L; IQR: 0.178-0.609 µg/L). S100B concentrations were significantly different between these three age-groups (p < 0.001).

#### **1 Population characteristics according to age** (Table 2)

2 For patients aged 65 to 80, median age was 73.5 years old (min: 65.0; max: 79.9; IQR: 69.4-76.9) and the sex ratio (M/F) was 1.1. For patients aged 80 to 90, median age was 85.1 years 3 old (min: 80.0; max: 89.9; IQR: 82.9-87.4) and the sex ratio (M/F) was 0.7. For patients aged 4 90 years and over, median age was 92.9 years old (min: 90.0; max: 105.1; IQR: 91.2-94.9) and 5 6 the sex ratio (M/F) was 0.5. The median time interval between mTBI and blood sample was not 7 significantly different between the three age-groups (p = 0.413). The proportions of S100B concentrations above the usual threshold of 0.10  $\mu$ g/L were 76.1%, 82.9%, and 90.2% for the 8 three age-groups (65-79, 80-89, and  $\geq$  90 years old), respectively (p < 0.001). The proportion 9 10 of positive CCT scans was not significantly different between the three age-groups (p = 0.11). Antiplatelet treatment was the most common risk factor: n = 272, n = 386, and n = 165 for the 11 three age-groups, respectively. 12

#### **S100B decisional threshold for older patients' management** (Table 3)

Of the 1172 patients included, 218 (18.6%) had a negative S100B ( $\leq 0.10 \ \mu g/L$ ). At the 0.10 14 15 µg/L threshold, S100B identified intracranial lesions observed in CCT scans with a sensitivity of 100% (95% CI: 94.3-100%), a specificity of 19.7% (95%CI: 17.4-22.1%) and a negative 16 predictive value of 100% (95% CI: 98.3-100%). For each age-group (65-79, 80-89, and  $\geq$  90 17 years old), specificity was 24.9% (95%CI: 21.0-29.1%), 18.2% (95%CI: 14.8-22.1%), and 18 10.5% (95%CI: 6.6-15.8%), respectively. A statistically significant difference was observed 19 when comparing the specificities obtained for each age-group (p < 0.001 between patients aged 20 65 to 80 and patients aged 90 years and over, p = 0.02 between patients aged 80 to 90 and 21 patients aged 90 years and over, and p = 0.01 between patients aged 65 to 80 and patients aged 22 80 to 90). 23

Optimal S100B thresholds (allowing the best specificity while keeping a 100% sensitivity) for each age group were determined and were as follows:  $0.11 \mu g/L$  for patients aged 65 to 80 and

0.15 µg/L for the others. For patients aged 65 to 80, the sensitivity was 100% (95%CI: 81.5-1 2 100%), the specificity was 28.4% (95%CI: 24.3-32.8%), and the negative predictive value was 100% (95% CI: 97.2-100%). For patients aged 80 to 90, the sensitivity was 100% (95%CI: 3 88.4-100%), the specificity was 34.3% (95%CI: 29.9-38.8%), and the negative predictive value 4 was 100% (95% CI: 97.7-100%). For patients aged 90 years and over, the sensitivity was 100% 5 (95%CI: 78.2-100%), the specificity was 20.5% (95%CI: 15.0-27.0%), and the negative 6 predictive value was 100% (95% CI: 91.0-100%). For each age-group, the specificity allowed 7 by the optimal thresholds was significantly higher than the one observed with a 0.10  $\mu$ g/L 8 threshold (28.4% vs 24.9%, 34.3% vs 18.2%, and 20.5% vs 10.5%; p < 0.001). 9 10 As the 0.15  $\mu$ g/L threshold was common to both age-groups (80-89 and  $\geq$  90 years old), performance was also assessed at this threshold for patients over the age of 80. In these 11 conditions, the sensitivity was 100% (95% CI: 92.1-100%), the specificity was 29.3% (95% CI: 12 13 25.9-33.0%), and the negative predictive value was 100% (95% CI: 98.1-100%). Furthermore, with the 0.15 µg/L threshold, the specificity for the 80-89 age group (34.3%) was significantly 14 15 higher than the specificity for the  $\geq 90$  years old age group (20.5%), (p = 0.001). **Influence of Dementia** (eTable 1) 16 17 In our cohort, a total of 279 patients were suffering from dementia. The proportion of patients with dementia increased significantly with age: 11.3% for the age group 65-79, 28.7% for the 18

age group 80-89, and 41.0% for the age group  $\ge 90$  (p < 0.001). Median S100B concentrations

20 were significantly different when comparing patients with dementia (0.301  $\mu$ g/L; min: 0.035;

21 max: 8.97; IQR: 0.159-0.568) and others (0.207 µg/L; min: 0.029; max: 5.40; IQR: 0.113-

22 0.415) (p < 0.001).

23 The performance of the optimal thresholds was re-evaluated in patients without dementia (n =

24 893). For patients aged 65 to 80, the specificity was 28.7% (95%CI: 24.3-33.3%) vs. 28.4%

25 (95%CI: 24.3-32.8%), (p = 0.92). For patients aged 80 to 90, the specificity was 37.5% (95%CI:

- 1 32.2-42.9%) vs. 34.3% (95%CI: 29.9-38.8%), (p = 0.36). For patients aged 90 years and over,
- 2 the specificity was 25.4% (95%CI: 17.7-34.4%) vs. 20.5% (95%CI: 15.0-27.0%), (p = 0.32).
- 3 The performance of the optimal thresholds was re-evaluated in patients with dementia (n =
- 4 279). For patients aged 65 to 80, the specificity was 25.9% (95%CI: 15.0-39.7%) vs. 28.4%
- 5 (95%CI: 24.3-32.8%), (p = 0.70). For patients aged 80 to 90, the specificity was 26.2% (95%CI:
- 6 18.8-34.6%) vs. 34.3% (95%CI: 29.9-38.8%), (p = 0.08). For patients aged 90 years and over,
- 7 the specificity was 13.2% (95%CI: 6.5-22.9%) vs. 20.5% (95%CI: 15.0-27.0%), (p = 0.16).

#### 1 **DISCUSSION**

2 The age effect on S100B blood concentrations was poorly investigated in previous studies. Children have higher levels of S100B concentrations than adults (20,21). However, 3 S100B levels in older mTBI patients are less known. In 2013, Calgagnile et al. showed that 4 S100B in older patients may be less effective regarding CCT scans reduction due to a very poor 5 specificity (22). In a recent study including 504 mTBI patients over 65 years old, we established 6 7 that these patients had higher serum S100B values than overall adult populations resulting in a low specificity of 18.7% (15). In order to study the age effect on S100B levels, we conducted 8 this study on a larger cohort of 1172 older patients. The decision algorithm (Figure 1) was not 9 applied by physicians for 127 (10.8%) patients. This observation is similar to the 14% described 10 by Calgagnile et al. (12). Our results confirm the low overall specificity (19.7%). We also 11 showed that S100B concentrations increase significantly with age. As expected, when using the 12 routine 0.10 µg/L threshold, specificity is lower in older patients: 24.9%, 18.2%, and 10.5% for 13 the age-groups 65-79, 80-89, and  $\geq 90$  years old, respectively. In order to regain specificity for 14 older patients ( $\geq 65$  years old), we propose new decisional thresholds based on ROC curves 15 16 analysis.

For patients aged 65 to 80, a 0.11 µg/L threshold provides a 28.4% specificity instead of 24.9% 17 with the 0.10 µg/L routine threshold. For patients aged 80 to 90, specificity increases 18 19 considerably with the new threshold of 0.15  $\mu$ g/L (18.2% to 34.3%), while sensitivity remains at 100%. For patients aged 90 years and over, a 0.15  $\mu$ g/L threshold enables a two-fold 20 specificity increase (10.5% to 20.5%) and a 100% sensitivity. Therefore, we propose the use of 21 22 a 0.15 µg/L threshold for routine management of patients aged 80 years and over. This threshold  $(0.15 \ \mu g/L)$  is particularly interesting, allowing a 29.3% CCT scan reduction which is similar 23 to the proportion in global adult population (~ 30%) (17–19). On the other hand, the threshold 24 of 0.11  $\mu$ g/L is less relevant for routine application due to the measurement uncertainty at this 25 concentration level (~ 6%), which can lead to confusion with the 0.10  $\mu$ g/L threshold. The 26

determination of age related S100B clinical decision thresholds is consistent with recently 1 2 published articles supporting the need for age-centered patients management (23). This is all the more important given the fact that older age is often included as a risk factor for 3 complications consecutive to mTBI. In mTBI clinical decision rules, patients' age is the only 4 demographic criterion used for risk stratification. Indeed, in most guidelines, patients aged over 5 65 years old are considered being at higher risk for having brain injury on CCT scan (5,6,14). 6 7 The SFMU's recommendations consider amnesia or loss of consciousness as an intermediate risk factor if the symptom is associated with any of the following factors: dangerous mechanism 8 of injury or patient's aged  $\geq 65$  years old (5). Moreover, antiplatelet medication intake is also 9 10 considered as an intermediate risk factor. Antiplatelet therapy is more common in older patients 11 (24).

Sheng et al. showed that cortical expression of S100B increases with age (25). S100B levels 12 13 measured in postmortem brain tissue of patients aged 60 years old and more without dementia, were higher than those observed in younger patients. However, all S100B levels remained 14 15 below those seen in Alzheimer's patients (25). In Alzheimer's disease, S100B overexpression is explained by the role played by activated astrocytes in the pathogenesis of  $\beta$ -amyloid plaques 16 17 (26). In mice, overexpression has been reported to exacerbate cerebral amyloidosis, which may 18 be a vicious circle (27). Several hypotheses can be formulated in order to explain S100B blood increase with aging (eFigure 1). First of all, increasing levels of  $\beta$ -amyloid plaques in the brain 19 may stimulate S100B synthesis as an attempt to regenerate neuronal damage (28,29). It has 20 21 been shown that S100B levels of older chronic schizophrenic patients are positively correlated with age and negatively with deficit symptoms. This suggests an impact of S100B in 22 neuroplasticity and aging (28). Moreover, neuronal architecture is altered with increasing age 23 while neuronal count does not change (30-33). A reduction in the complexity of dendrites 24 arborization and dendritic length is observed, and spine numbers are also decreased (33,34). As 25

a result, astrocytes may be recruited for dendrites regrowth by releasing trophic factors 1 2 (31,35,36) like S100B protein. Neuroinflammation characteristic of aging ("inflammaging") 3 may also activate astrocytes cells (35,37,38). Astrocyte reactivity may then lead to an exaggerated release of astrocytic biomarkers like GFAP (32,37) and potentially S100B. 4 Astrocytes have a duality in their phenotype, neurotoxic or neuroprotective (37,39). While 5 astrocytes lose their neuroprotective functions in Alzheimer's disease (37), molecular changes 6 7 upon physiological aging are more complex. During normal aging, the impact of astrocyte activation and reactive gliosis may be neuroprotective at first (39). Experimental data from 8 9 selected central nervous system (CNS) pathologies suggest that if not resolved in time, reactive 10 gliosis can exert inhibitory effects on neuroplasticity and CNS regeneration (39), which is consistent with S100B effects. Nanomolar concentrations of S100B exert neurotrophic effects 11 by stimulating neurite outgrowth and regulating survival of neurons whereas micromolar 12 13 concentrations are neurotoxic for the neuronal environment (40). Another hypothesis for the increased S100B blood levels observed in older adults is an increased BBB permeability. A 14 15 meta-analysis reported a significant increase in BBB permeability in old healthy subjects (n = 238) when compared to younger healthy subjects (n = 213) (41). Increased S100B blood levels 16 17 could also be linked to an extracerebral release since this protein is also expressed in other 18 tissues such as skeletal muscle or bone tissue (8). A recent study showed an S100B increase in muscle cells in patients with sarcopenia (42). Another study showed higher S100B blood levels 19 in patients with acute fractures without apparent cerebral injury (43,44). A higher incidence of 20 21 sarcopenia and fractures in older people could also explain the S100B increase in this population (42,45). Furthermore, kidney function, which is known to decrease with age, may 22 also contribute to the elevation of the S100B protein itself eliminated by the kidney (46,47). 23 However, Jönsson et al. showed that the elimination of S100B was not affected by a moderate 24 reduction of glomerular filtration (48). This could be explained by the low molecular weight of 25

S100B (21 kDa) allowing it to be easily filtered by the renal glomeruli. Lastly, in patients with
end-stage renal disease, chronic inflammation and dysfunction of the BBB could increase the
release of S100B by astrocytes and thus potentially explain the complication of cognitive
impairment well known in these patients (49).

TBI results in astrocyte release of S100B, which may itself contribute to brain damage. 5 Herrmann et al. showed, on a group of patients with mTBI, that increased posttraumatic 6 7 concentrations of S100B help to identify the patients with a risk of long term neuropsychological dysfunction (50). In addition, S100B appears to be an important and useful 8 predictor of functional outcome in moderate-to-severe TBI (51). An increase of S100B in 9 10 traumatic brain conditions (micromolar levels) may contribute to brain damage by promoting 11 inflammatory activities in astrocytes that cause neuronal dysfunction (52,53). This observation is all the more relevant because older people have an exaggerated astrocytic reactivity with 12 higher basal levels of S100B, which explains why post-traumatic concentrations more easily 13 reach micromolar levels. Perhaps this is why the TBI can contribute to dementia (54). Aging 14 15 also seems to alter the mechanisms of cerebral compensation, plasticity and cerebral reorganization of the brain (55). Moreover, older patients often have more difficulty making the 16 required psychological adjustments to the traumatic event. Globally, S100B levels could in part 17 18 explain the less favourable outcome for elderly patients (55).

Finally, we questioned the effect of Alzheimer's disease and other dementias on S100B concentrations. As expected, median S100B concentrations are significantly different between patients with dementia (0.301 µg/L) and others (0.207 µg/L) (p < 0.001). The high rate of dementia among patients over 90 years of age (~ 41.0%) in our study population is consistent with the estimated prevalence of dementia in France (56). In 2018, 40.8% of people over 90 years old had dementia (56). In our cohort, the optimal threshold of 0.15 µg/L would have avoided 20% of CCT-scans in people over 90 years old against 10% with the routine threshold

of 0.10 µg/L. The exclusion of patients with dementia could enable a 25% CCT scans reduction 1 2 instead of 20% when these were included. Although there is no statistically significant difference between the two values (p = 0.32), a 25% specificity is closer to the one in average 3 adult populations (~ 30%) and the AUC for this subgroup is not significant. In addition, we 4 obtain lower specificity values in dementia patients compared to our whole cohort. For patients 5 aged 90 years and over, the specificity falls from 20% to 13%. Once again, the difference is not 6 7 significant (p = 0.16), which proves the necessity for a new study focused on dementia patients 8 to revaluate optimal thresholds.

9 Recently, similar studies in older patients have also shown a reduced specificity of serum Glial 10 Fibrillary Acidic Protein (GFAP) (23,57) and Ubiquitin Carboxy-terminal Hydrolase L1 (UCH-11 L1) (23). Only Tau protein has been shown to have equivalent specificity performance between different age-groups (57). Another study, presenting data on GFAP and Neurofilament Light 12 (NFL) levels in the cerebrospinal fluid of healthy participants, showed an increase in both 13 biomarkers with increasing age (58). Finally, these data point to the influence of older age on 14 15 candidate mTBI biomarkers. As for S100B, thresholds' adjustment will be necessary in older patients' management. Due to the retrospective nature of the study, we were unable to measure 16 17 any brain biomarkers other than S100B. These assays will be the subject of future work.

in some patients. Moreover, clinical data on dementia was collected retrospectively, and the etiology of dementia could not be identified for all patients. Some information (such as the time between the trauma and the blood sample) provided by patients with dementia may be questionable. In addition, future work will be required to achieve a better pathophysiological understanding of blood S100B increase in older patients. Finally, the retrospective nature of the study limited us in collecting data such as co-morbid conditions (e.g. chronic kidney disease,

One of the limitations of the study was the lack of direct ascertainment of intracranial lesions

estimated glomerular filtration rate (eGFR) reduction, sarcopenia, recent bone fractures) or
 medication intake (glucocorticoids, other anti-inflammatory drugs, ...).

In conclusion, S100B levels are considerably affected by age. At the routine threshold (0.10  $\mu$ g/L), its clinical utility is very limited by a poor specificity leading to only a small decrease in CCT scanning. Adjustment of the S100B threshold is necessary in older patients' management. An optimal 0.15 µg/L threshold is particularly interesting for patients over 80 years of age with  $a \sim 30\%$  CCT scans reduction, which is comparable to the CCT scans reduction allowed in relatively younger adults. To validate the accuracy of our results, a future prospective study will be needed.

### 10 Conflict of Interest

11 The authors have no conflicts.

### 12 Funding

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#### 1 <u>TABLE LEGENDS</u>

2 Table 1: S100B concentration levels according to age.

3 IQR: interquartile range; max: maximum; min: minimum. These data include the entire
4 population (CCT performed or not) (n = 1172).

5 Table 2: Population characteristics according to age.

AT : antiplatelet treatment; CCT: cranial computed tomography; CCT+: ≥ 1 intracerebral
lesion; F: females; h: hours; IQR: interquartile range; LC: loss of consciousness; M: males;
max: maximum; min: minimum; RA: retrograde amnesia; †: time between trauma and sample;
\*: p value of a multivariate model including gender.
Table 3: Analysis of sensitivity (SE), specificity (SP), positive predictive value (PPV) and
negative predictive value (NPV) for the two thresholds tested on the three age categories.

- 12 AUC: area under ROC curve; yo: years old; \*: Optimal threshold (keeping 100% SE): 0.11
- 13  $\mu$ g/L for patients aged 65 to 80, 0.15  $\mu$ g/L for patients aged 80 to 90, and 0.15  $\mu$ g/L for patients
- 14 aged 90 years and over;  $\dagger$ : Comparison of the specificity obtained at the 0.10  $\mu$ g/L threshold to
- 15 that obtained at the optimal threshold.

## **Table 1:** S100B concentration levels according to age

2

Age-group	n	S100R median ug/L (min: may: LOP)				
(years old)		Sivob incuran, µg/i	<i>p</i> value			
<mark>65-69</mark>	138	0.183				
	160	0.187	0.184			
<mark>70-74</mark>		(0.038; 3.77; 0.105-0.360)	(0.029; 7.74; 0.103-0.360)			
<mark>75-79</mark>	178	0.182				
		(0.029; 7.74; 0.109-0.343)				
<mark>80-84</mark>	239	0.235 (0.035; 5.40; 0.129-0.468)	0.255	< 0.001		
<mark>85-89</mark>	252	0.266 (0.032; 8.97; 0.128-0.486)	(0.032; 8.97; 0.129-0.474)			
<mark>90-94</mark>	154	0.317 (0.039; 3.52; 0.168-0.608)	0.318			
≥ 95	51	0.331 (0.086; 4.68; 0.179-0.700)	(0.039; 4.68; 0.178-0.609)			

3

4 IQR: interquartile range; max: maximum; min: minimum.

5 These data include the entire population (CCT performed or not) (n = 1172).

## **Table 2:** Population characteristics according to age

	<mark>65-79</mark> years old	<mark>80-89</mark> years old	$\geq$ 90 years old		
	n = 476	n = 491	n = 205	<i>p</i> value	
Age median, years	73.5	85.1	92.9	< 0.001	
(min; max; IQR)	(65.0; 79.9; 69.4-76.9)	(80.0; 89.9; 82.9-87.4)	(90.0; 105.1; 91.2-94.9)		
Sex ratio M/F	1.1	0.7	0.5	< 0.001	
Median time <sup>†</sup> , h	1.7	1.5	1.7	0.412	
(min; max; IQR)	(0.3; 3.0; 1.2-2.2)	(0.2; 3.0; 1.2-2.0)	(0.3; 3.0; 1.3-2.0)	0.413	
S100B median, µg/L	0.184	0.255	0.318	< 0.001*	
(min; max; IQR)	(0.029; 7.74; 0.103-0.360)   (0.032; 8.97; 0.129-0.474)   (0.039; 4.68; 0.178)		(0.039; 4.68; 0.178-0.609)	< 0.001	
%S100B+	76.1	82.0	90.2	< 0.001*	
threshold of 0.10 $\mu$ g/L	/0.1	62.7	70.2	< 0.001	
%S100B+	72 7	67.8	81.0	< 0.001*	
optimal threshold	12.1	07.0	01.0		
CCT + (%)	n = 18	n = 30	n = 15	0.11	
	(1.5%)	(2.6%)	(1.3%)	0.11	
<b>Risk factor</b>					
- RA (%)	n = 49 (4.2%)	n = 27 (2.3%)	n = 20 (1.7%)	0.017	
- LC (%)	n = 233 (19.9%)	n = 151 (12.9%)	n = 44 (3.8%)	< 0.001	
- AT (%)	n = 272 (23.2%)	n = 386 (32.9%)	n = 165 (14.1%)	< 0.001	
- ≥ 2 (%)	n = 71 (6.1%)	n = 67 (5.7%)	n = 24 (2.0%)	0.532	

AT : antiplatelet treatment; CCT: cranial computed tomography; CCT+: ≥ 1 intracerebral
lesion; F: females; h: hours; IQR: interquartile range; LC: loss of consciousness; M: males;
max: maximum; min: minimum; RA: retrograde amnesia; †: time between trauma and sample;
\*: p value of a multivariate model including gender.

- 1 Table 3: Analysis of sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) for the two thresholds
- 2 tested on the three age categories.

	AUC	Threshold of 0.10 μg/L				Optimal threshold*				
	(95% CI)	SE	SP	PPV	NPV	SE	SP	PPV	NPV	<i>p</i> value <sup>†</sup>
		(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)	
All patients	0.71	100	19.7	6.6	100	/	/	/	/	/
Ĩ	(0.66-0.76)	(94.3-100)	(17.4-22.1)	(5.1-8.4)	(98.3-100)					
65-79 vo	0.71	100	24.9	5.0	100	100	28.4	5.2	100	< 0.001
<b>03-72</b> 90	(0.61-0.82)	(81.5-100)	(21.0-29.1)	(3.0-7.7)	(96.8-100)	(81.5-100)	(24.3-32.8)	(3.1-8.1)	(97.2-100)	
80 80 vo	0.75	100	18.2	7.4	100	100	34.3	9.0	100	< 0.001
<del>00-02</del> y0	(0.68-0.82)	(88.4-100)	(14.8-22.1)	(5.0-10.4)	(95.7-100)	(88.4-100)	(29.9-38.8)	(6.2-12.6)	(97.7-100)	> 0.001
> 00 vo	0.55	100	10.5	8.1	100	100	20.5	9.0	100	< 0.001
≥ 90 yo	(0.45-0.65)	(78.2-100)	(6.6-15.8)	(4.6-13.0)	(83.2-100)	(78.2-100)	(15.0-27.0)	(5.2-14.5)	(91.0-100)	~ 0.001

3 AUC: area under ROC curve; yo: years old; \*: Optimal threshold (keeping 100% SE): 0.11 µg/L for patients aged 65 to 80, 0.15 µg/L for patients

4 aged 80 to 90, and 0.15 μg/L for patients aged 90 years and over; †: Comparison of the specificity obtained at the 0.10 μg/L threshold to that

5 obtained at the optimal threshold.

#### 1 **<u>FIGURE LEGENDS</u>**

- 2 Figure 1: Decision algorithm for patients' management with mild traumatic brain injury
- 3 (mTBI) at the Clermont-Ferrand Emergency Department. The reference threshold is

## 4 internationally defined as 0.10 μg/L for adults.

- 5 CCT: Cranial Computed Tomography; GCS: Glasgow Coma Scale; h: hours; LOC: Loss Of
- 6 Consciousness.
- 7 Figure 2: Flow chart of patients included, S100B protein results and CCT results.
- 8 CCT: Cranial Computed Tomography; GCS: Glasgow Coma Scale; mTBI: mild Traumatic
- 9 Brain Injury; S100B-: serum concentrations below the cutoff (< 0.10  $\mu$ g/L); S100+: serum
- 10 concentrations above the cutoff ( $\geq 0.10 \ \mu g/L$ ); ø CCT: CCT not performed.



Figure 1



