Background: The place of serum S100B measurement in mild traumatic brain injury (mTBI) management is still controversial. Our prospective study aimed to evaluate its utility in the largest child cohort described to date.

Methods: Children younger than 16 years presenting at a pediatric emergency department within 3 h after TBI were enrolled prospectively for blood sampling to determine serum S100B concentrations. The following information was collected: TBI severity determined by using the Masters classification [1: minimal or Glasgow Coma Scale (GCS) 15, 2: mild or GCS 13–15, and 3: severe or GCS <13]; whether hospitalized or not; good or bad clinical evolution (CE); whether cranial computed tomography (CCT) was prescribed; and related presence (CCT+) or absence (CCT−) of lesions.

Results: For the 446 children enrolled, the median concentrations of S100B were 0.21, 0.31, and 0.44 μg/L in Masters groups 1, 2, and 3, respectively, with a statistically significant difference between these groups (P < 0.05). In Masters group 2, 65 CCT scans were carried out. Measurement of S100B identified patients as CCT+ with 100% (95% CI 85–100) sensitivity and 33% (95% CI 20–50) specificity. Of the 424 children scored Masters 1 or 2, 21 presented “bad CE.” S100B identified bad CE patients with 100% (95% CI 84–100) sensitivity and 36% (95% CI 31–41) specificity. Of the 242 children hospitalized, 81 presented an S100B concentration within the reference interval.

Conclusions: Serum S100B determination during the first 3 h of management of children with mTBI has the potential to reduce the number of CCT scans, thereby avoiding unnecessary irradiation, and to save hospitalization costs.

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Accidents are the major cause of morbidity and mortality in children, and head trauma is the injury most often responsible for death. Case series from multiple trauma centers report that 75% to 97% of trauma deaths in children result from head injuries (1). The incidence of traumatic brain injury (TBI)4 is calculated as 1/285 in babies younger than 1 year (2), and up to 80% of deaths in children younger than 2 years are due to inflicted TBI. A study of severe fatal TBI in the US found incidence figures similar to those for the UK, at approximately 1/3300 children age 0–12 months (3). Patients with minimal or severe TBI are easy to recognize in clinical practice, but the correct diagnosis of mild TBI (mTBI) in babies and younger children is difficult even for experienced physicians, partly because questioning rarely provides a good history of trauma, and partly because infants present common nonspecific clinical symptoms. Current policy addressing this issue hinges on 2 strategies: routine cranial computed tomography (CCT) and discharge in the absence of symptoms, and inpatient observation for up to 24 h and CCT in the event of clinical deterioration. Both approaches present drawbacks. CCT is associated with exposure to ionizing radiation and sedation, which is required during the examination in infants and young children, whereas observation is costly. Accordingly, algorithms for the evaluation and triage of children and adolescents with mTBI were set up (4, 5) to reduce unnecessary CCT and inpatient observation. Even so, most CCT and short hospitalization can still be potentially avoided, because 93% of children with mTBI have no intracerebral lesions (6). An economic...
strategy was developed that was based on the use of biomarkers to support a reduction in unnecessary care, and there is a clear trend toward increased international efforts to evaluate the use of biomarkers in pediatric TBI (7–9). Many blood biomarkers have been proposed and evaluated in this clinical context, but S100B protein appears to be the most sensitive and relevant.

S100B is a small dimeric protein (molecular weight approximately 21 kDa) that consists of ββ or αβ chains and belongs to a multigenic family of calcium-binding proteins (10). Predominantly expressed by central nervous system cells (mainly astroglial but also neuronal cells), S100B can also be detected in melanoma cells and to some extent in other tissues. This functional protein is involved in a variety of intracellular and extracellular regulatory activities, including protein phosphorylation, cell motility, and neuronal differentiation and proliferation (11). For mTBI, the advantage of using the S100B biomarker in adults is well established (12–14). Undén et al. have confirmed this utility in a metaanalysis, and voiced the need for more studies in children (15). The role of this marker in the initial assessment of children with mTBI is still controversial and ill defined (16). In a study of 109 children, Castellani et al. (8) showed that S100B with a cutoff threshold that gives a sensitivity of 100% reduces unnecessary CCT. Hallen et al., who did a study on 111 children, suggested that S100B could be a valuable diagnostic tool added to those used in clinical practice today (17). Betchel et al. have drawn a similar conclusion and highlighted the need for further studies with more inclusion (18). For all of these studies the reports mention the need to establish reference intervals for serum S100B protein during the first 3 years of life; these were recently established by our group (19).

In this context, the aim of our study was to evaluate the utility of serum S100B measurement in mTBI management on a large cohort of children by evaluating the significance of serum S100B concentration differences in children with closed head trauma divided into 3 groups on the basis of TBI severity (minimal, mild, and severe) and by evaluating the ability of this biomarker to help the physician reduce both unnecessary CCT and short hospitalization for observation.

Materials and Methods

STUDY DESIGN AND PATIENTS

This prospective study was carried out from April 2010 to April 2011 in the Department of Paediatric Emergency of Clermont–Ferrand Hospital, after approval by the South-East Ethics Committee of France (reference number JV-TV/2010–15) and in compliance with the Declaration of Helsinki ethical principles for medical research involving human study participants. Informed consent was obtained from all parents or legal guardians. All children (age 0–16 years, admission within 3 h) with closed head trauma were eligible for enrollment and were ranked in 3 severity groups according to the Masters classification (20). Masters group 3 (severe TBI), which formed a positive control group, was composed of children with a Glasgow Coma Scale (GCS) <13 or loss of consciousness or progressive decrease in consciousness. Masters group 2 (mTBI) comprised children with a GCS score of 13–15 on admission and 1 or more of 12 clinical risk factors: brief loss of consciousness, posttraumatic amnesia, nausea, vomiting, severe or progressive headache, dizziness, vertigo, intoxication, anticoagulation, skull fracture, seizure, age <2 years. Masters group 1 (minimal TBI) was made up of children with a GCS score of 15 without symptoms or with only headache or bruising. Pregnant women, children whose TBI occurred >3 h before presentation, and multiply injured patients were excluded. Once a patient was enrolled in the study, a venous blood sample was drawn and multiply injured patients were excluded. Once a patient was enrolled in the study, a venous blood sample was drawn and the decision whether to order a CCT scan and/or hospitalization in Masters 2 patients was made by the physician in the emergency department, respecting the classical management without the indication of S100B concentrations done after the end of all clinical inclusions. Anamnestic and demographic information was noted. Clinical evolution (CE) was appraised over 24 h. Bad CE was defined by the following clinical symptoms: vomiting, facial paralysis, movement disorders, vertigo, photomotor reflex disorder, seizure, progressive headache, or behavior change. Good CE was defined as the absence of these symptoms. The nonhospitalized patients were followed for 24 to 48 h after consultation, with a standardized telephone interview led by a unique clinical research associate. For all the children information for the following items was collected: frequency of vomiting since returning home; problems or difficulty the parents had observed the child having in moving an arm or a leg; convulsions; any changes in facial expression; parental opinion of child’s return to the previous state health before the consultation or what changes had been observed. For the children over 4 years old, additional information was collected regarding complaints of headaches.

CRANIAL COMPUTED TOMOGRAPHY

An emergency CCT scan was performed with the following protocol: 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 divided into 2 groups: CCT-negative (CCT−) mTBI patients with no signs of traumarelevant intracerebral lesions and CCT-positive (CCT+) mTBI patients with at least 1 pathophysiological trauma–relevant intracerebral lesion.

SERUM S100B MEASUREMENT
Venous blood samples were processed to serum and deep frozen at −80 °C until assayed after being checked for stability during the freezing period. We determined serum S100B concentrations with an electrochemiluminescence immunoassay on a Roche Diagnostics Modular Analytics system E170 instrument. Each measurement was performed in duplicate according to the manufacturer’s recommendations (21). The S100B calibration curve was linear up to 39 μg/L, and the CV for duplicates across the entire concentration range for calibrators and samples was 3.1%. According to the manufacturer the lower limit of detection of the assay was 0.005 μg/L. Values of S100B had no effect on the clinical management of the patients. Recently established reference intervals (19) were used: the upper serum S100B reference limits (95th percentile) were derived for 3 age groups: 0.35 μg/L for age 0–9 months, 0.23 μg/L for age 10–24 months, and 0.18 μg/L for age >24 months. Patients exhibiting serum concentrations below the specific age-range cutoff were counted as S100B negative (S100B−), and those with concentrations above as S100B positive (S100B+).

STATISTICS
All statistical tests were performed with SAS 9.1.3 software (SAS Institute). Because the demographic and epidemiological data were not normally distributed, they are reported as median and interquartile range. Comparisons of S100B values among Masters groups 1, 2, and 3 were performed with a nonparametric Kruskal–Wallis test followed by the post hoc Dunn multiple comparisons procedure to determine whether the S100B concentrations varied in Masters groups from 1 to 3. We evaluated the difference in S100B+ proportion in each Masters group with a Fisher test followed by a Cochran–Armitage test for trend. For subgroup analysis, we used a U-test to compare the S100B concentrations between patients with trauma-relevant intracerebral lesions (CCT+) with those in patients without such lesions (CCT−). The S100B concentrations between bad CE and good CE groups were also compared. The sensitivity, specificity, and positive and negative predictive values for the defined cutoff level were determined in these 2 contexts. Patients were evaluated in accordance with their serum S100B concentrations as S100B+ or S100B−, and this discrimination was cross-checked against results of the CCT scan (CCT+ or CCT−), CE (bad or good CE), and whether the patients were hospitalized. For all these values, we calculated the 95% CI using the normal approximation method. To determine the discriminative ability of S100B serum measurements in Masters group 2 (mTBI) patients, 2 ROC curves were calculated according to the dichotomous variables CCT− or CCT+ and bad or good CE.

Results

PATIENTS AND SERUM S100B MEDIAN CONCENTRATIONS
Prospectively, 446 children with TBI were enrolled, comprising 183 Masters 1 (41%), 241 Masters 2 (54%), and 22 Masters 3 (5%) patients. The median interval between trauma and blood sampling was 2 h 05 min (range 1 h 30 min to 2 h 45 min or 25%–75%). The sex ratio (male/female) was 1.68. The median age was 5.2 years (range 2.1–9.9 or 25%–75%). Of these 446 children 77% were >2 years old, 17% between 9 and 24 months old, and 7% <9 months old. Domestic accident was the main cause of TBI (73%), most commonly owing to a fall from child’s height (27%) and then a fall between 1 and 2 m (19%) (Table 1). The median concentrations of S100B were 0.21 (interquartile range 0.15–0.29), 0.31 (range 0.18–0.47), and 0.44 μg/L (range 0.30–0.66) in Masters groups 1, 2, and 3, respectively. The difference across these 3 groups was statistically significant (P < 0.05) (Fig. 1). In addition, a significant linear (Cochran–Armitage test for trend; P = 0.013) increase in the S100B+ percentage was ob-

<table>
<thead>
<tr>
<th>Table 1. Origins of traumatic brain injury for the 446 children.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
</tr>
<tr>
<td>Domestic accident</td>
</tr>
<tr>
<td>Fall from child’s height</td>
</tr>
<tr>
<td>Fall between 1 and 2 m</td>
</tr>
<tr>
<td>Fall while running</td>
</tr>
<tr>
<td>Fall down stairs</td>
</tr>
<tr>
<td>Collision</td>
</tr>
<tr>
<td>Fall &gt;2 m</td>
</tr>
<tr>
<td>Sports related</td>
</tr>
<tr>
<td>Bicycle, ski, or horse</td>
</tr>
<tr>
<td>Collision</td>
</tr>
<tr>
<td>Road accident</td>
</tr>
<tr>
<td>Bicycle, pedestrian</td>
</tr>
<tr>
<td>Scooter, motorcycle</td>
</tr>
<tr>
<td>Car</td>
</tr>
<tr>
<td>Intoxication/discomfort</td>
</tr>
<tr>
<td>Not specified</td>
</tr>
</tbody>
</table>
served according to the Masters group: 60% in Masters group 1, 70% in Masters group 2, and 100% in Masters group 3 (Fig. 1).

**S100B, CE, AND ECONOMIC UTILITY**

Of the 424 Masters 1 or 2 children, 21 were included in the bad CE group. As shown in Table 2, measurement of S100B identified patients correctly as bad CE with a sensitivity of 100% (CI, 84%–100%) and a specificity of 36% (CI, 31%–41%). With an area under the curve value of 0.75 (CI, 0.70–0.79) in the ROC analysis (Fig. 2), S100B measurement was found to be a highly significant indicator for the correct stratification of patients with TBI into the bad CE or good CE groups (area under ROC curve = 0.75; *P* = 0.0001). The best threshold conserving a sensitivity of 100% was 0.19 μg/L. Of the 242 hospitalized children (Masters 1 and 2), 81 (33.5%) were S100B+. An S100B assay costs US$44 and daily hospitalization in France costs US$1587. Hence a potential saving of US$117 875 [(81 nonhospitalized × US$1587) − (242 potentially hospitalized patients × US$44)] could be made with no harm to patients.

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**Table 2. S100B concentration by CCT, clinical evolution, and management.**

<table>
<thead>
<tr>
<th>S100B+</th>
<th>S100B−</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT+</td>
<td>23</td>
<td>0</td>
<td>100% (85.2%–100%)</td>
<td>33% (20%–50%)</td>
<td>45% (31%–60%)</td>
</tr>
<tr>
<td>CCT−</td>
<td>28</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad CE</td>
<td>21</td>
<td>0</td>
<td>100% (84%–100%)</td>
<td>36% (31%–41%)</td>
<td>8% (5%–11%)</td>
</tr>
<tr>
<td>Good CE</td>
<td>258</td>
<td>145</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized</td>
<td>161</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonhospitalized</td>
<td>118</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Patients exhibiting serum concentrations below the cutoff (0.35 μg/L for age 0–9 months; 0.23 μg/L for age 10–24 months; 0.18 μg/L for age >24 months) were counted as S100B−, and those above as S100B+. CCT+: mTBI patients with no signs of trauma-relevant intracerebral lesions. CCT−: mTBI patients with at least 1 pathophysiological trauma-relevant intracerebral lesion. The symptoms of bad CE were vomiting, facial paralysis, movement disorders, vertigo, photomotor reflex disorder, seizure, progressive headache, or behavior change. Good CE was indicated by absence of these symptoms. Numbers in parentheses are 95% CIs.
ROC curve, 0.72 (95% CI, 85.2%–100%); \( P = 0.002 \). The best threshold conserving a sensitivity of 100% was 0.18 \( \mu g/L \).

**Discussion**

Our prospective study, based on 446 children, the largest cohort used to date, demonstrated the potential interest of determining blood concentrations of S100B protein for the management of children with mTBI. The utility of S100B is already well established for adults. Incorporation of S100B concentrations into the clinical decision rules for CCT in adult mTBI patients could reduce the total number of negative CCT scans by as much as 30% (12–15). For the first time in a pediatric context, a linear increase in serum S100B concentration according to the severity of the TBI was observed. This is a first argument justifying the utility of this biomarker for children with mTBI. As established in adults (12, 14) and in children (8), our results show that the inclusion of S100B concentration with use of a cutoff threshold that provides a sensitivity of 100% in the clinical decision rules for CCT in mTBI patients could reduce the total number of negative CCT scans by as much as 33% with a sensitivity of 100%. Importantly, these results could allow irradiation during pediatric mTBI management to be reduced. If the lifetime attributable risk of a fatal cancer from 1 CT examination is plotted as a function of age, the risks are greatest by far for an infant and decline rapidly with age (22).

Although CCT remains the actual gold standard, clinical practice shows that physicians prefer short hospitalization for observation over radiological examinations. These hospitalizations are indicated for Masters 2 children but also for some Masters 1 if parents are very worried or unable to provide home monitoring. For the first time, our results demonstrate that blood assay of S100B in Masters groups 1 and 2 correctly identified patients with bad CE with a sensitivity of 100% and a specificity of 36%. S100B measurement in patients with TBI was found to be a highly statistically significant indicator for the correct stratification of each patient into the bad CE or good CE group (area under the ROC curve of 0.75; \( P = 0.0001 \)). The best thresholds conserving a sensitivity of 100% to identify bad CE and CCT+ children were 0.19 and 0.18 \( \mu g/L \), respectively. These concentrations are similar to the reference interval that we recently defined for children older than 2 years (19), the age group representing 77% of our current cohort.

The correct identification of patients as CCT+ and as bad CE by S100B, with no harm to patients, shows that inclusion of S100B measurement in the clinical decision criteria for children with mTBI could reduce the total number of unnecessary hospitalization.
by 33.5% with a sensitivity of 100%. On the other hand, a decision to hospitalize children with only a S100B+ result would cause an increase in unnecessary hospitalization. Like many nonspecific biomarkers, serum S100B assay must not be used alone but be combined with clinical examinations for assessment and triage of children and adolescents with mTBI, as proposed in an alternative management scheme, which will have to be demonstrated and compared to the established one in a future multicenter study (Fig. 4). Because S100B has a short half-life of 120 min (23), it is important to specify in the proposed future algorithm that a venous sample must be taken within 3 h after the TBI. In a recent review it was reported that delayed serum measure-
ments of S100B following a head injury may lead to erroneous conclusions regarding the extent of neuro-
nal damage (16). Reduction in blood sampling volume should be encouraged, according to the recent publication regarding capillary sampling for measurement of S100B, a collection procedure that is more easily performed and minimizes pain and discomfort for the child (24). This proposed strategy based on S100B could also be strengthened in the future by the addition of new markers such as ubiquitin C-terminal hydrolase-L1 and all-spectrin breakdown product 145 kDa, which do not increase after mild pediatric trauma brain injury (25), and by further confirmatory studies of our decisional algorithm with a high number of samples for the positive controls (CTT+ and bad CE). Although this number of CTT+ and bad CE samples is relatively low in the current study, our results appear promising, owing to the fact that the deduced sensitiv-
ity and specificity are strongly similar to those obtained with a high number of patients (8, 13).

In conclusion, we have demonstrated the utility of S100B protein determination in serum for the manage-
ment of children with mTBI. After validation in a multi-
center study that includes a high number of cases with abnormal CCT and bad CE, this determination could avoid unnecessary irradiation, produce hospital cost savings, and reduce the duration of mTBI management in pediatric emergency departments.

**Fig. 4.** Established and proposed (with use of S100B measurement) decision algorithm for CCT or hospitalization indication in children under mTBI management.

The text written in black (with solid frames and arrows) represents the current practice of mTBI management. The gray text (with dotted frames and arrows) represents an alternative decisional algorithm including S100B measurement.

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