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Clinical commentary

Plasma micro-RNA biomarkers for diagnosis and prognosis after traumatic brain injury: A pilot study

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ABSTRACT

Prediction of post-concussive syndrome after apparent mild traumatic brain injury (TBI) and subsequent cognitive recovery remains challenging, with substantial limitations of current methods of cognitive testing. This pilot study aimed to determine if levels of micro ribonucleic acids (RNAs) circulating in plasma are altered following TBI, and if changes to levels of such biomarkers over time could assist in determination of prognosis after TBI.

Patients were enrolled after TBI on presentation to the Emergency Department and allocated to three groups: A – TBI (physical trauma to the head), witnessed loss of consciousness, amnesia, GCS = 15, a normal CT Brain and a recorded first pass after post-traumatic amnesia (PTA) scale; B TBI, witnessed LOC, amnesia, GCS = 15, a normal CT brain and a PTA scale test fail and: C – TBI and initial GCS <13 on arrival to the ED. Venous blood was collected at three time points (arrival, day 5 and day 30). Isolation of cell-free total RNA was then assayed using a custom miRNA PCR array.

Two micro-RNAs, mir142-3p and mir423-3p demonstrated potential clinical utility differentiating patients after mild head injury into those at greater risk of developing amnesia and therefore, post-concussive syndromes. In addition, these miRNA demonstrated a decrease in expression over time, possibly indicative of brain healing after the injury. Further evaluation of these identified miRNA markers with larger patient cohorts, correlation with clinical symptoms and analysis over longer time periods are essential next steps in developing objective markers of severity of TBI.

1. Introduction

Country-based incidence of traumatic brain injury (TBI) estimates range from 108 to 332 hospitalised new cases per 100,000 population per year. The incidence is rising as a consequence of increased transport-related injuries in low and middle-income countries [1,2]. TBI is classified as mild, moderate or severe, depending on the patient's presenting level of consciousness as expressed by the Glasgow Coma Scale (GCS) score [3,4]. Persons with GCS scores of 3–8 are classified with a severe TBI, those with scores of 9–12 are classified with a moderate TBI, and those with scores of 13–15 are classified with a mild TBI.

Among patients with moderate to severe head injury, early prognosis is important to direct management options and guide prognosis. A combination of the GCS score along with clinical variables such as pupillary reaction and computed tomography (CT) brain findings are utilised by most clinicians, but self-assessed to be accurate only 37% of the time [5].

Up to 15% of patients with mild TBI experience persistent disabling problems, including reduced functional ability, heightened

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emotional distress, and delayed return to work or school [6]. In the state of Victoria, Australia, there were about 500 reported hospitalisations per year for sports-related concussions alone [7]. Existing tools to assess the extent of mild TBI include the Galveston Orientation and Amnesia Test (GOAT), the (Modified) Oxford PTA Scale (MOPTAS), the Westmead post-traumatic amnesia (PTA) Scale (WPTAS), Sports Concussion Assessment Tool version 3 (SCAT-3) [8] and the Revised-WPTAS (2004, Ponsford version) [9–12]. Limitations associated with these scales refer to imperfect accuracy, because not all answers to memory questions can be verified [13]. Furthermore, several test items are retrospective in nature, and pictures are used instead of words as memory items, which may be impractical, especially in Emergency Department (ED) settings and the level of task difficulty with some questions routinely failed by control subjects [14].

In recent years, plasmatic and cerebrospinal fluid biomarkers have emerged as possible tools to distinguish between the different pathophysiological processes after TBI [15]. A number of biomarkers of injury to different cell types and structures within the brain can be detected in peripheral blood, but utility and detection have been limited due to altered expression secondary to the blood–brain barrier and paucity of clinical evaluation [16,17]. Among these, S100-B and glial fibrillary acidic proteins have been demonstrated to be increased in patients with TBI and correlate with Glasgow Coma Scale scores and neuro-radiological findings at hospital admission [18].

Micro ribonucleic acids (miRNAs) are a large class of endogenous, small, single-stranded non-coding molecules composed of 20–24 nucleotides that regulate gene expression at the post-transcriptional level [19]. They are small, unique molecules that bind to messenger RNAs (mRNA) and inhibit the production of proteins. In many cases, miRNAs are upregulated in neuropathological conditions such as Alzheimer's and Parkinson's disease. miRNAs may be important mediators of the profound molecular and cellular changes that occur after TBI in both the short and the long term.

Microarray analyses in rat models have revealed both dynamic temporal regulation of miRNA expression within the cortex, with the numbers of downregulated and upregulated miRNAs peaking at 24 h and 72 h post-injury, respectively [20]. Furthermore, the most frequent sequelae of TBI is associated with hippocampal pathology manifesting as cognitive and memory impairments, and microarray studies on animal models of TBI have revealed significant changes in miRNA expression within the hippocampus [21,22]. As a biomarker of pathology, miRNAs have several unique characteristics including cell- and tissue- and disease-specific expression patterns [23–25]. They are extremely stable, they are small enough to cross the blood brain barrier and enter the bloodstream, and are now easily detected by emerging testing methods.

This pilot study aimed to determine if the levels of miRNAs circulating in plasma are altered following mild to severe TBI, and if changes to levels of such biomarkers over time could be used to assist in determination of prognosis after TBI.

2. Methods

2.1. Setting

The state of Victoria, Australia has one paediatric and two adult Major Trauma Services (MTS) located within metropolitan Melbourne. The Alfred Hospital ED is an adult major trauma centre, which receives approximately 60 000 presentations per year.

2.2. Patient selection

This study was approved by The Alfred Hospital Research and Ethics Committee. (Project ID 486/14). This was a prospective, single-centre, non-randomised, cohort pilot study [26]. A convenience sample of patients presenting at a time when study investigators were present in the ED was enrolled. Inclusion and exclusion criteria are listed in Table 1. All patients after mild TBI undergo post-traumatic amnesia testing at this hospital, prior to discharge from the ED. The Westmead Post-traumatic Amnesia Scale (WPTAS) is used and is a brief bedside standardised test that measures length of PTA after TBI. It consists of twelve questions to assess orientation and ability to consistently retain new information. Previous evaluation of the WPTAS has been found to have high inter-rater reliability and predictive validity [27,28]. Informed consent was obtained from the person responsible and patients were sub-grouped into three cohorts:

Group A: Patients presenting with a TBI (physical trauma to the head), witnessed LOC, amnesia, GCS = 15, a normal CT Brain and a first abbreviated-WPTAS pass.

Group B: Patients with TBI, witnessed LOC, amnesia, GCS = 15, a normal CT brain and a first abbreviated-WPTAS fail. This sub-group of patients were deemed to be at high risk of developing a post-concussive syndrome. However, identification of such patients during initial assessment can be difficult and this study aimed to determine differences among biomarker levels among patients in Group A and Group B.

Group C: Patients with TBI and GCS <13 on arrival to the ED, to select patients with moderate or severe brain injury. This sub-group of patients can be readily identified during initial clinical assessment. Biomarker levels in this group were measured to confirm that levels correspond to severity of injury. In addition, levels in all 3 groups were tested at 3 time-points to assess if changes reflected the healing process.

The enrolling study investigator collected initial blood samples and prospectively recorded clinical and injury variables in a pre-abstracted form (Table 1). A low fall was defined as fall from standing height or from an elevation of less than 1.0 m. The shock index is defined as heart rate divided by systolic blood pressure [29]. Acute traumatic coagulopathy was defined by an International Normalised Ratio (INR) $\geqslant$1.3 in the absence of oral anticoagulant use [30].

2.3. Blood sample collection

Venous blood was collected from each patient at three time points (arrival, day 5 and day 30). Following baseline blood sample, subsequent blood was collected on day 5 and day 30 post-injury following the standard hospital procedures. In the event that the patient was discharged or did not return for a therapeutic visit for the days 5 and 30 collection times, study investigators travelled to patients' residence or rehabilitation facility to collect the

Table 1
Inclusion and exclusion criteria.

Inclusion criteria:
1. Adult patients (age $\geqslant$18 years)
2. Injured through any mechanism- blunt or penetrating
3. Initial GCS = 15 with a normal CT brain or GCS <13
4. Venous blood sample can be collected within 4 h upon reaching the hospital
5. Patients with isolated head injury, as determined on initial assessment
6. LOC has to be recorded as witnessed, not reported by the patient alone
7. The period of amnesia may be variable and may range from amnesia of the event only to a longer time frame
8. Person responsible available to provide informed consent

Exclusion criteria:
1. Venepuncture in patient not feasible
2. Nursing home residents
3. Pregnancy