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The diagnostic utility of salivary microRNAs in non-athlete concussion

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DECLARATION

The majority of the following work described in this essay has been completed by Ms Emma Toman, under the guidance of Prof Antonio Belli and Dr Valentina Di Pietro. The CONTACTS study was designed and delivered by Ms Toman, as was the lab work and statistical analyses. The work was undertaken entirely in the UK.

DEDICATIONS AND THANKS

This work would not have been possible without the help and guidance of the following people: Prof Belli and Dr Di Pietro- as my mentors and PhD supervisors at the University of Birmingham, Prof Welbury, Mr Hammond, Lt Col Naumann, Ms Cooper, the Oral and Maxillofacial Surgery, Trauma and Orthopaedic Surgery and Emergency Departments at University Hospitals Birmingham, and all the members of the former Surgical Reconstruction and Microbiology Research Centre.

The following essay is dedicated to my eternally supportive husband and children. Thank you.

ABSTRACT

Background

Concussion accounts for 1.2% of *all* Emergency Department (ED) attenders in England and Wales and is frequently missed. There is currently no objective, diagnostic test for concussion and identifying the disease is often complicated by concurrent intoxication, psychiatric illness and pre-existing cognitive comorbidities. Micro-RNA (miRNA) has recently emerged as a promising biomarker for sports-related concussion (SRC) but are these findings translatable into the NHS?

The aim of this essay is to provide a brief overview of concussion and to present the initial findings from the CONTACTS study, assessing the translatability of existing SRC assessment tools and investigating the diagnostic utility of salivary miRNA in the ED.

Methods

CONTACTS was an exploratory cohort study comparing 9 patients with concussion against 9 control patients with isolated limb trauma. Saliva samples were taken in the ED and tested for a predetermined panel of miRNA based on results from previous sports research. In addition, the Sports Concussion Assessment Tool (SCAT5) and the Immediate Post Concussion Assessment Tool (ImPACT) Quick tests were completed.

Following normalisation, independent t-tests were performed using the ΔC_T values of concussed vs trauma control and fold change in miRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Results

The concussed cohort demonstrated significant upregulation of hsa-let-7f (p .024) and downregulation of hsa-miR-144-3p and hsa-miR-34b-3p (p .004 and .022 respectively). Area under curve values for hsa-let-7f and hsa-miR-144-3p were statistically significant and demonstrated excellent diagnostic accuracy (0.82 and 0.88 respectively).

Neither the SCAT5 or ImPACT Quick tests were able to discern between the concussed and control cohorts.

Conclusion

Results from the SCAT5 and ImPACT Quick assessments do not support their use in the Emergency Department. However, salivary miRNAs show diagnostic promise and warrant further investigation in larger pragmatic studies within our NHS population.

BACKGROUND

Definition of concussion

Widely used colloquially, the term *concussion* has been more readily adopted by the medical community over recent years. In 2023 the International Concussion in Sport Group (CISG) updated their definition of sports-related concussion (SRC) to include the most up-to-date evidence relating to concussion pathophysiology. The group defined concussion as a "traumatic brain injury induced by biomechanical forces," highlighting several common defining features: (1)

1. Injury causes an impulsive force to be transmitted to the head
2. Initiation of a neurotransmitter and metabolic cascade, with possible axonal injury, blood flow change and inflammation affecting the brain
3. Symptoms and signs presenting immediately, or evolving over minutes or hours, and commonly resolving within days, but may be prolonged
4. No abnormality is seen on standard structural neuroimaging studies
5. Results in a range of clinical symptoms and signs that may or may not involve loss of consciousness
6. The clinical syndrome cannot be explained solely by (but may occur concomitantly with) drug, alcohol or medication use, other injuries, or comorbidities.

Previously, the term *mild traumatic brain injury (mTBI)* was preferred by clinicians (2) but recent recommendations from the American Congress of Rehabilitation Medicine (ACRM) have suggested the term mTBI may be used synonymously with concussion. (3)

Epidemiology of concussion

mTBI accounts for 1.2% of all Emergency Department (ED) attenders in England and Wales. (4) The actual number of patients experiencing mTBI however is predicted to be much higher. Concussion frequently occurs alongside extracranial injury too, with 40% of patients with maxillofacial injury and almost 70% of major trauma patients suffering a concurrent mTBI. (5, 6)

As with most trauma, young men and older women are at the highest risk of suffering concussion. (7, 8) Falls from standing, assault and road traffic collisions (RTC) are the most common mechanisms of injury. (5, 9) Concurrent comorbidities and intoxication are common

(10) with 30% of all mTBI patients suffering psychiatric illness, and >70% of older patients having history of premorbid circulatory disease. (11, 12) Multiple psychosocial factors are more prevalent in concussed patients including, low cognitive function and social deprivation. (13, 14) Of note, 82% of prisoners have reported sustaining a previous TBI and of these 59% are classified as mild. (15)

Pathophysiology

Ionic flux and neurotransmitter release

Immediately following impact, shearing and stretching forces cause neuronal potassium efflux and calcium influx resulting in unregulated depolarisation. (16, 17) The resultant excitatory neurotransmitter, glutamate, release and compromised gamma-amino-butyric-acid (GABA) interneuron activity allows for exaggerated feedback loops of hyperexcitability and further depolarisation. (18) See figure 1.

Metabolic demand

In the battle to restore neuronal membrane potential following injury, there is increased activity of the sodium-potassium (Na^+-K^+) pump. The metabolic demand of the cell therefore increases. When the glucose supply can't keep up with demand, anaerobic metabolism takes over resulting in an accumulation of acidic intracellular lactate. (18) Meanwhile, increased intracellular calcium sequesters in the mitochondria leading to mitochondrial impairment and further metabolic imbalance. (19)

This intracellular energy crisis is compounded by compromised cerebral blood flow and feeble oxidative breakdown. Rendering the post concussive brain more vulnerable to further damage and less able to respond adequately to a second injury. (20-23)

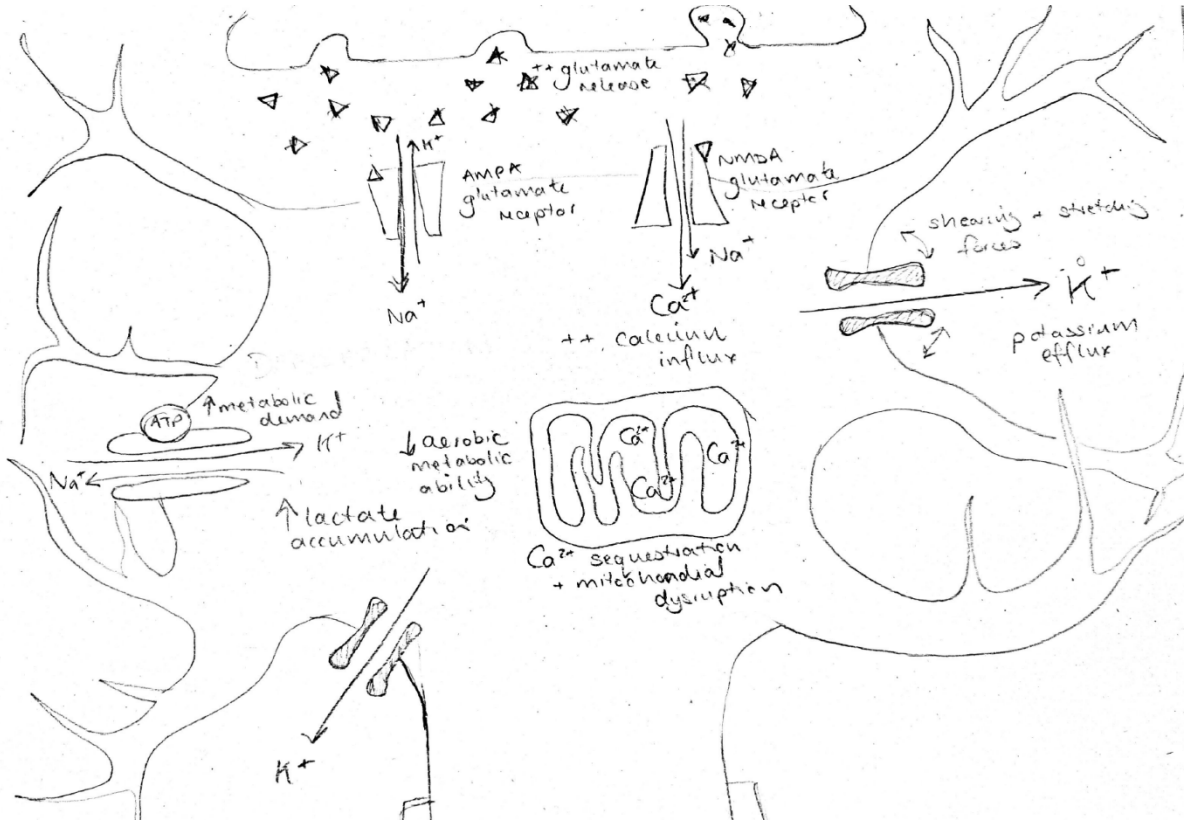

Figure 1. ionic and metabolic changes following concussion

Axonal and cytoskeletal injury

Whilst no structural injury is visible using standard neuroimaging, microstructural trauma has been identified to play a role in concussion pathophysiology. Neurofilament and microtubule distortion occurs directly from trauma and secondary to high intra-axonal calcium. This disturbs axonal transport and allows the accumulation of beta-amyloid precursor protein (b-APP) leading to axonal swelling. (24, 25)

Cerebral perfusion and blood-brain-barrier

Research into cerebral perfusion and BBB disruption in concussion is limited by the lack of invasive monitoring that can be used for more severe disease. However, researchers have hypothesised that the classic triphasic (hypoperfusion, hyperaemia, and vasospasm) response seen in severe TBI also occurs in mTBI. (26)

The blood-brain barrier (BBB) regulates CNS communication with the periphery through physical barriers, transport, secretions, and metabolic blockade. (27) In the hours and days following TBI, expression of junctional adhesion proteins decreases causing greater vessel

permeability leading to increased leakage of plasma proteins and resultant cerebral oedema. (28) A delayed second phase of BBB dysfunction may occur days later and has been demonstrated to take days to weeks to recover. (29-31) Persistent BBB permeability has been identified in 73% of patients with a diagnosis of post-concussion syndrome. (32)

Neuroinflammation

Neuroinflammation in response to concussion has both beneficial and detrimental effects. It occurs via resident glia activation, release of inflammatory cytokines and recruitment of peripheral leukocytes. (33, 34) Microglia produce anti-inflammatory mediators, scavenge cellular debris, and choreograph the inflammatory response, yet also produce pro-inflammatory molecules that exacerbate secondary brain injury. (35) When the BBB is interrupted, circulating immune cells and plasma proteins capable of inducing neuroinflammation and cerebral vascular dysfunction (including fibrinogen) are more able to extravasate into the brain. (36-38) Persistent neuroinflammation may linger for years and has been hypothesised to correlate with symptom burden and duration. (39)

Diagnosis of concussion

Identifying a concussion is important as early, simple interventions following injury reduce the likelihood of post-concussive syndrome (PCS) developing. (40) Currently there is no objective diagnostic test for concussion which proves a major challenge for diagnosis in NHS patients. Advanced imaging, genetic testing and biomarkers are important research tools, but they are yet to prove clinically useful.

The current gold standard for concussion diagnosis is thorough history and examination. The clinical picture of mTBI can be broadly subdivided into: symptoms, physical signs, balance impairment, behavioural changes, cognitive impairment, and sleep/wake disturbance (1) and are detailed in figure 2.

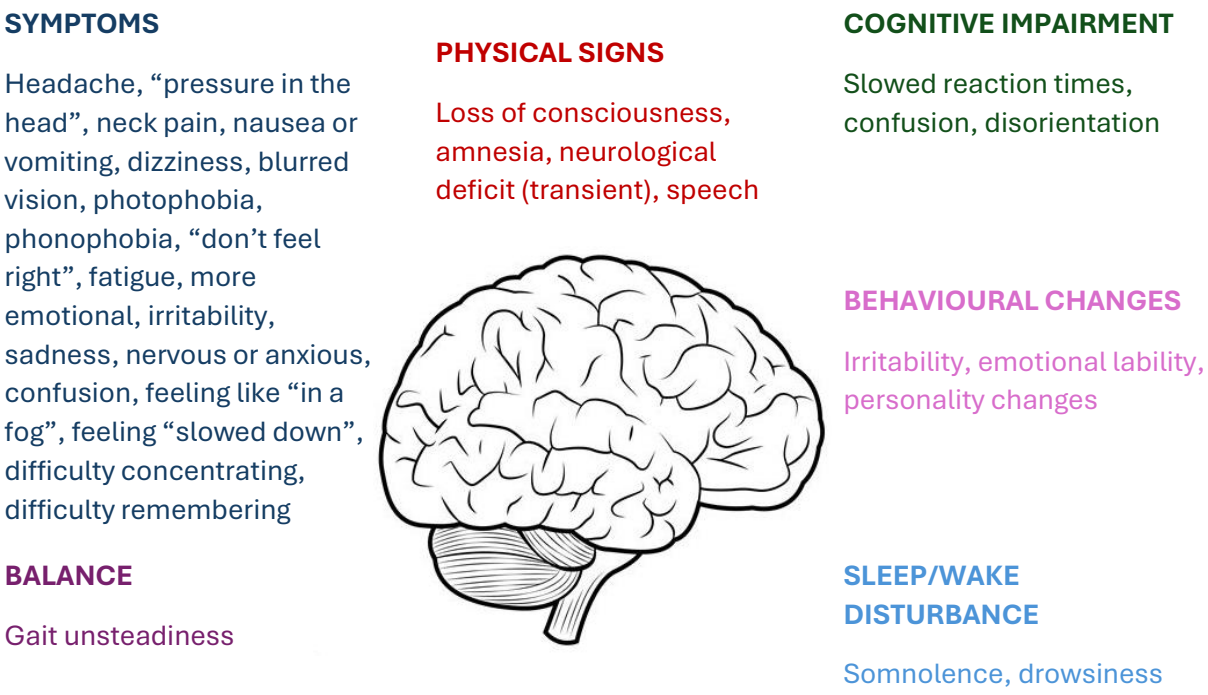

Figure 2. Clinical presentation of concussion

Diagnosis is straightforward when individuals fall limp to the ground with loss of consciousness (LOC) following head injury, however, this is complicated when the injury is unwitnessed, insidious, without LOC, symptoms are delayed or there is co-existing cognitive deficit or intoxication. Moreover, mTBI investigation is often side-lined when there are concurrent “physically obvious” injuries. For example, over half of patients with a spinal cord injury have a missed TBI and most of these are concussions. (41)

One third of patients with mTBI in ED have concurrent intoxication (4) but the CISG definition of concussion states that “clinical signs and symptoms cannot be explained by drug, alcohol, or medication use.” (1) How then, are clinicians to diagnose mTBI satisfactorily in these patients? The CISG also state that “clinical signs and symptoms cannot be explained by other injuries (such as cervical injuries, peripheral vestibular dysfunction, etc) or other comorbidities (such as psychological factors or coexisting medical conditions),” (1) leaving older people and those suffering with cognitive or psychiatric disease more vulnerable to a missed concussion diagnosis.

Both the standard MRI brain protocol (T1, T2, Diffusion Weighted Imaging (DWI), Susceptibility Weighted Imaging (SWI) and Fluid-Attenuated Inversion Recovery (FLAIR) sequences) and CT head show no abnormality in patients with concussion, as per CISG criteria. Advanced MRI

techniques such as Diffusion Tensor Imaging (DTI), Magnetic Resonance Spectroscopy (MRS), Functional Magnetic Resonance Imaging (fMRI) and Arterial Spin Labelling (ASL) are used extensively in research to better understand pathophysiology but are of little use in clinical diagnostics.

Sports-related concussion assessments

The Sports Concussion Assessment Tool (SCAT), now on version 6, combines symptom, orientation, memory, concentration, and balance with clinical neurological examination for assessment of SRC. (42) Recent investigation into its use in non-athletes however showed minimal success in distinguishing concussed from control patients in a controlled environment. (43, 44)

Whilst neuropsychological assessment is vital in the management of concussion, the cost and logistical implication of every suspected concussion patient having access to a clinical neuropsychologist is insurmountable. In professional sports, the use of computerised neuropsychological assessments has been widely adopted. The most commonly used is the Immediate Post Concussion Assessment Tool (ImPACT) which scores verbal memory, visual memory, reaction time, processing speed and impulse control. (45)

Biomarkers

Discovery of an objective, diagnostic biomarker is the holy grail of concussion management. Ideally, a biomarker of concussion should reflect the level of neuronal injury correlating to brain function and outcome in a linear fashion. Currently the only clinically utilised biomarker for TBI is S-100β, following its incorporation into Scandinavian Neurotrauma Committee TBI guidelines in 2013. (46)

MicroRNAs (miRNAs) are a class of small endogenous non-coding RNAs, around 22 nucleotides in length. (47) These molecules regulate gene expression at a post-transcriptional level by binding to messenger RNA (mRNA) in the cytoplasm and encourage mRNA degradation or prevention of translation. In response to injury or disease, miRNA can be either under or overexpressed. Where under expressed, the lack of inhibiting miRNA influence leads to increased translation of its paired gene. Where overexpressed, the abundance of inhibitory miRNA leads to decreased translation of said gene. In this way >60% of human protein-coding genes are under the control of miRNAs. (48)

Traditionally, biomarkers have reflected damage to a specific tissue to indicate a disease process e.g. troponin in myocardial infarction or S-100β in moderate-severe TBI. Changes in miRNAs however reflect the modulation of a biological response to insult. The main challenge

this poses for their use to diagnose concussion is that responses such as neuroinflammation and metabolism are dynamic and so levels of miRNA are difficult to interpret depending on the amount of time elapsed since injury.

Concussion-specific circulating serum miR425-5p was first discovered in 2017 (49) and whilst serum miRNA shows promise for a diagnostic concussion test, it was felt impractical for the purposes of "pitch side" testing of professional athletes. Saliva has since been suggested as a potential source of miRNA. The most significant results so far come from the SCRUM study in 2021 in which a panel of 14 salivary miRNAs successfully identified concussed rugby players from matched controls. The panel was able to differentiate between clinically diagnosed concussion and clinically excluded concussion immediately post-match and at 36-48 hours. This has significant implications for use in professional sports and potentially for non-athletes in the ED. (50) Salivary miRNAs are worthy of further investigation in the non-athlete setting where there is a far greater variation in age, physical and cognitive baseline characteristics of patients presenting with head injury.

The aim of *Concussion in Non-athletes: Assessment of Cognition and Symptomatology (CONTACTS)* study (51) was to investigate the utility of both existing SRC assessments and salivary miRNA to identify concussion in a sample of NHS patients. The following methods and results are the initial findings from the CONTACTS study.

METHODS

The study protocol for the CONTACTS study is available online. (51) This was a single-centre prospective cohort study and was approved by the West Midlands - Coventry & Warwickshire Research Ethics Committee (ref 20/WM/0299). As part of the CONTACTS study, there were several follow up timepoints following initial recruitment and assessment the ED- *these data/results however are not included as part of this essay*.

The study was designed in consultation with the local Patient and Public Involvement and Engagement (PPIE) group- the Trauma Advisory Group.

Participants

Patients of interest included adult patients suffering maxillofacial trauma and concurrent concussion who required hospital admission. Recruiting patients with maxillofacial trauma to the concussion arm ensured objective evidence of head injury having occurred.

The trauma control cohort included patients with isolated limb trauma and no evidence of head injury requiring admission. Patients with isolated limb injury were chosen as a suitable control group because they have a comparable burden of injury and receive similar management to the concussed group such as operative interventions and pain management.

Including patients requiring admission aimed to reduce loss-to-follow-up rate at the 24-48h follow up timepoint. Detailed inclusion and exclusion criteria are included as part of figure 3.

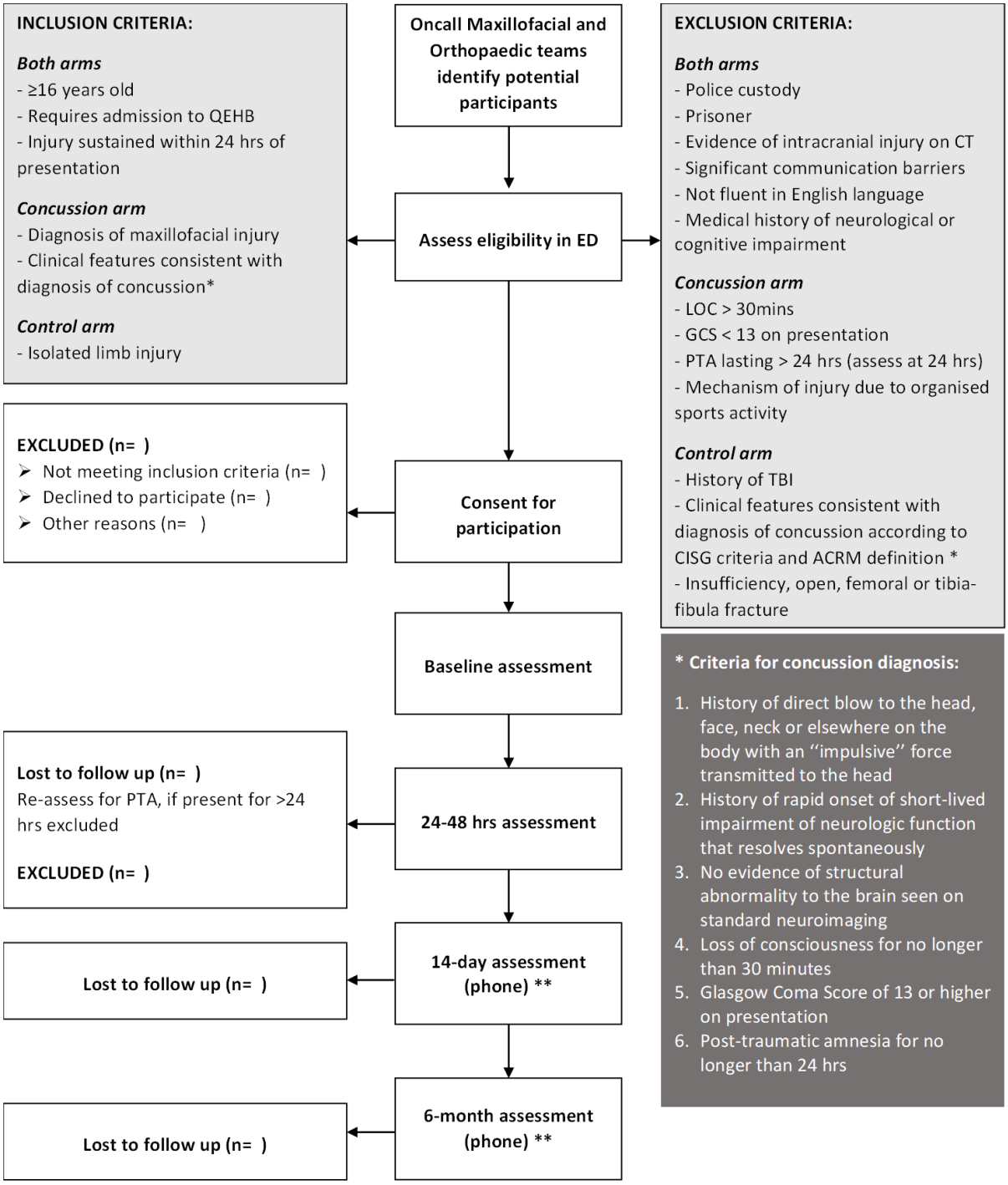

Figure 3. recruitment flow chart for CONTACTS study

Baseline and study assessment data

All participants had a medical history and clinical examination as part of routine standard of care. In addition to a saliva sample, further study-related data were collected following recruitment:

- ImPACT Quick assessment (3 composite scores)
- SCAT5 assessment (6 domains scored)
- Educational level (number of years of education completed)
- Level of intoxicated (number of units of alcohol in the past 12 hours)
- Diagnosis of learning disability or attention deficit hyperactivity disorder (ADHD)

As previously mentioned, the follow up time points beyond this initial assessment in the ED are not included as part of this essay.

SCAT5 assessment

The SCAT5 was developed through "a systematic review and synthesis of current research, public input and expert panel review" as part of the 5th International Consensus Conference on Concussion in Sport held in Berlin in 2016". (52) The SCAT5 is validated for assessment of sports-related concussion in patients 13 years or older and should take no less than 10 minutes to perform. The assessment should be conducted by healthcare professionals only and is not designed to be a standalone tool in the diagnosis of concussion. (52) Table 1 summarises the separate elements of the SCAT5 assessment.

Table 1. Summary of SCAT5 domains

Step	Description	Scoring
Red flag	List of red flag signs and symptoms of intracranial or cervical spine injury	No score
Observable signs	Descriptions of observations of patient following injury (either witnessed or video observation)	No score
Memory assessment	"Maddock's questions" aim to assess the patient's orientation to time and place	No score
Examination	Glasgow Coma Score and cervical spine assessment	No score
Athlete background	Demographic, educational, concussion, mental health and neurological history	No score
Symptom evaluation	22 symptoms with ability to grade from "none" to "severe"	Symptom number (of 22) Symptom severity score (of 132)
Cognitive screening (Standardised Assessment of Concussion)	Tasks aimed to assess orientation, immediate memory and concentration.	Orientation (of 5) Immediate memory (of 15 or 30) Concentration (of 5)
Neurological screen	Gross neurological assessment of vision, co-ordination and gait. Includes "modified balance error scoring system"	Normal or abnormal Balance errors (of 30)
Delayed recall	Whether the patient is able to recall words previously listed in immediate recall	Delayed recall (of 5 or 10)
Decision	This section allows for documentation of each of the above domain scores and immediate management plan	No score

All scored domains were used in the CONTACTS study other than balance as the majority of trauma control patients had a lower limb injury.

ImPACT Quick assessment

The ImPACT tool is a computer-based neurocognitive assessment widely used across a variety of professional sports. (53) The ImPACT Quick is a condensed version of the test that can be performed in 5-7 minutes and is administered using a tablet. (54) It includes 6 subtests of the full

version to give 3 composite scores for speed, memory and attention. Each composite score is determined through combination of results from a series of cognitive tests. The full ImPACT scoring requires a pre-injury assessment to which post-concussion scores are compared and a Reliable Change Index (RCI) is calculated to identify abnormal results- clearly not applicable for NHS patients. (55) The ImPACT Quick however uses percentile scores derived from comparison to normative values from a large representative sample in place of pre-injury. (56)

As with SCAT, the ImPACT has been researched in several non-athlete studies with various conclusions, usually highlighting significant differences in only a few subsets. (57-61) In addition to the limited inclusion criteria for SCAT studies, previous studies into ImPACT have actively excluded anyone over the age of 60 as the tests are not validated outside of this range. The manner of testing in these studies is also not obtainable in everyday clinical use as most studies used a separate, quiet room in the ED where patients were not disturbed during the assessment.

miRNA analysis

Saliva samples were collected in OCR-100 saliva collection pots containing a proprietary miRNA stabilising solution. Samples were stored in -80 degrees freezer and analysed in batches once recruitment was complete. miRNA profiles were analysed using TaqMan™ technology for miRNA extraction and analysed using QIAGEN sequencing software.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test prior to analyses. Age was analysed using the t-test, all other continuous data using the Mann-Whitney test. Categorical data were analysed using Fisher's Exact test or X^2 where appropriate.

Normalisation of the comparison threshold (C_T) was performed by calculating the difference between the housekeeping assay mean C_T and the assay C_T (miRNA of interest). After normalisation, 20 was added to the normalised ΔC_T values to shift the numbers in a positive range to allow using the qPCR analysis pipelines according Qiagen procedures. Following normalisation, independent t-tests were performed using the ΔC_T values of concussed vs trauma control.

Fold change in miRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method. (62) Using this method the data are presented as the fold change in miRNA expression normalised to the chosen housekeeping assay (hsa-let-7b) and relative to the trauma controls.

Software used was GraphPad Prism 10.0.2 and statistical significance threshold set at $p < .05$.

RESULTS

Demographic data are presented in table 2 and demonstrate no significant difference between cohorts. Similarly clinical data are presented in table 3, showing only a significant difference between the number of CT head scans performed between groups- as expected.

Table 2. Basic demographic data for participants

	Concussed (n=9)	**Trauma control (n=9)**	**p value**
Age (years)	29.0 (16-48)	38.2 (20-68)	.170
Sex	22.2 (2)	33.3 (3)	> .999
Qualification level			
None	11.1 (1)	11.1 (1)	.753
GCSE/equivalent	55.6 (5)	55.6 (5)	
A level	33.3 (3)	22.2 (2)	
Undergraduate degree	0 (0)	11.1 (1)	
Post-graduate degree	0 (0)	0 (0)	

Age displayed as mean (range), sex displayed as % female (n), qualification displayed as % (n)

Table 3. Clinical data for participants

	Concussed (n=9)	Trauma control (n=9)	p value
Relevant PMHx			
Psychiatric diagnosis	3	1	
Epilepsy	2	0	
Learning difficulties	1	1	.413
Previous concussion	4	2	
Substance abuse	1	1	
Other	0	2	
Analgesics received since injury			
Simple analgesia	7	9	
Weak opioid	2	8	.354
Strong opioid	6	9	
Other	1	0	
Mechanism of injury			
Assault	5	0	
RTC	2	2	
Fall <2m	1	2	.081
Fall >2m	1	1	
Other	0	3	
No. of alcohol units in last 12h	0.22 (0-2)	0.00 (0-0)	> .999
No. of CTH performed	0.89 (0-1)	0.0 (0-0)	**< .001**
GCS on arrival	15 (15-15)	15 (15-15)	> .999

Relevant PMHx displayed as no. of diagnoses per cohort- some participants may have more than one diagnosis. No. of alcohol units displayed as mean (range). CTH displayed as mean (range) no. of CTs performed per pt. GCS displayed as mean (range).

The injuries diagnosed within the trauma control group included: 5 tibial fractures, 3 ankle fractures and 1 humerus fracture. The injuries diagnosed within the concussed group included: 6 mandible fractures, 1 Le Fort 3 fracture, 1 zygoma fracture and 1 facial laceration.

There were some missing data points. One participant was unable to complete the SCAT5 assessment in the ED as they were taken to theatre prior to the test being administered. Six participants were unable to complete the ImPACT Quick assessment however for the following reasons: 3 x internet unavailable, 1 x pt taken to theatre before test completed, 1 x iPad unavailable and 1 x dominant arm injury.

ImPACT Quick and SCAT5

Results showed no statistically significant difference in ImPACT Quick composite scores between the concussed and control cohorts- results are presented in table 4. There were also no significant differences between cohorts of the six SCAT5 domains, as shown in table 5.

Table 4. comparison of composite scores for ImPACT Quick in the ED

Composite	Concussed	Trauma control	U	p value
Speed	14.40 (28.00)	30.57 (50.00)	13.00	.514
Memory	18.40 (29.50)	27.43 (48.50)	14.50	.669
Attention	48.00 (35.00)	40.43 (43.00)	15.00	.753

Concussed and trauma control values are displayed as mean (sum of ranks)

Table 5. Comparison of SCAT5 domains in the ED

SCAT5 domain	Concussed	Trauma control	U	p value
Symptom number	11.25 (77.50)	11.00 (75.50)	30.50	.620
Symptom score	44.63 (74.50)	38.44 (78.50)	33.50	.835
Orientation	4.57 (56.50)	4.67 (79.50)	28.50	> .999
Immediate recall	12.57 (54.00)	13.44 (82.00)	26.00	.598
Concentration	2.14 (48.50)	3.00 (87.50)	20.50	.260
Delayed recall	3.17 (48.00)	2.75 (57.00)	21.00	.825

Concussed and trauma control values are displayed as mean (sum of ranks)

Salivary miRNA

Table 6 presents the results of fold change comparison between concussed and trauma control groups in the ED. The concussed cohort demonstrated significant upregulation of hsa-let-7f (p .024) and downregulation of hsa-miR-144-3p and hsa-miR-34b-3p (p .004 and .022 respectively).

Area under curve values for hsa-let-7f and hsa-miR-144-3p were statistically significant and demonstrated excellent (63) diagnostic accuracy (0.82 and 0.88 respectively).

Table 6. Univariate analysis of ED timepoint concussion (Con) vs trauma control (TC)

Con vs TC	AUC	95% CI	mean ΔC_T Con	mean ΔC_T TC	$\Delta\Delta C_T$	Fold change Con/TC	t-test p-value
hsa-let-7a	0.77	[0.45, 1.00]	-9.16	-8.18	-0.98	0.51	.166
hsa-let-7f	**0.82***	[0.58, 1.00]	-12.93	-14.96	2.03	4.12	**.024***
hsa-miR-21-5p	0.52	[0.25, 0.78]	-18.90	-18.27	-0.63	0.65	.597
hsa-miR-103a-3p	0.70	[0.44, 0.95]	-16.03	-17.31	1.28	2.43	.210
hsa-miR-144-3p	**0.88***	[0.71, 1.00]	-10.20	-7.07	-3.13	0.11	**.004***
hsa-miR-34b-3p	0.78	[0.52, 1.00]	-14.42	-12.24	-2.19	0.22	**.022***
hsa-miR-135b-5p	0.62	[0.34, 0.89]	-16.33	-15.77	-0.56	0.68	.439
hsa-let-7i	0.80	[0.52, 1.00]	-18.52	-17.04	-1.48	0.36	.229
hsa-miR-16-1-3p	0.72	[0.47, 0.96]	-12.68	-11.19	-1.49	0.35	.092

*Denotes statistical significance where p< .05

AUC Area Under Curve, C_T comparative threshold, Con concussed group, TC trauma control group

DISCUSSION

The primary findings of the CONTACTS study demonstrate that whilst the SCAT5 and ImPACT Quick tests cannot discriminate concussed from control patients in the ED, several salivary miRNAs show diagnostic promise.

Fewer participants had missing data in the SCAT5 test compared to the ImPACT Quick. The SCAT5 is very simple to deliver and is a paper-based assessment freely available online making it very accessible for clinicians. (42) By contrast, the ImPACT Quick requires a paid licence, access to a tablet and working internet- all three pulling on resources scarce within the average NHS Emergency Department.

SCAT5 in the ED

Almost all previous studies using the SCAT are, unsurprisingly, in young athletes. Several studies concerning non-athletes have been published however with mixed conclusions. (64, 65) The main drawback to non-athletes studies however are limited inclusion criteria that frequently exclude patients with concurrent learning disability, psychiatric illness, intoxication or premorbid cognitive disorders. For translation into the ED, research must address these patient cohorts especially as several studies have demonstrated that SCAT scores are significantly affected by such factors. (66, 67)

In the CONTACTS study no domains of the SCAT5 were significantly different in the concussed cohort compared to the trauma control cohort. We postulate this is because the majority of symptoms following concussion are non-specific and are readily found in patients suffering painful limb trauma who have been awake for hours under bright ED lights in a noisy department.

Our findings however are in in contrast to several previous studies. A US study in which mTBI patients had significantly poorer scores in both the symptoms checklist and standardised concussion assessment (SCA) compared to healthy controls and orthopaedic trauma controls. (64) However, only patients who had received a CT head were recruited into this study. To reach criteria for CT scanning following a head injury, red flag symptoms of TBI must be present in the first place, heavily influencing symptoms checklist and SCA. The study also excluded patients

over the age of 60 in addition to any patients having consumed illicit drugs or alcohol in the previous 24 hrs.

ImPACT Quick in the ED

Similarly to the SCAT5, the ImPACT Quick assessment was not able to discern concussed from control patients in our study sample. In comparison to the full ImPACT assessment, the shorter ImPACT Quick has been included in far fewer published works. There have been no studies until now investigating its potential use in non-athletes.

A recent literature review examining the validity of the ImPACT test revealed that although the tool demonstrated sound convergent validity, research describing discriminant validity and diagnostic accuracy was either inconclusive or scanty. (57) Very few of the studies included in the review concerned the use of ImPACT in non-sports populations and only three of the sixty-nine studies analysed the use of ImPACT in concussed versus orthopaedic controls.

The most comparable study to CONTACTS was completed in 2017 in the USA in which 94 concussed and 80 matched-trauma controls were recruited from the ED. The participants completed the ImPACT (not ImPACT Quick) assessment within 72 hours of injury, 15 days and 45 days and no significant difference in composite scores were found between groups at any of the time points. (68) Once again, highlighting the need to include a wider range of concussed patients in research, the US study limited its participant inclusion criteria to patients under the age of 45 years.

In contrast to CONTACTS and the aforementioned US study, an Australian study administered the ImPACT assessment to 79 mTBI patients and 86 trauma control patients in the ED and found significant differences in all 5 composite domain scores between the two groups. (60) However, the study recruited no participants over the age of 61 and also controlled for blood alcohol concentration. Although this study highlights the potential utility of the ImPACT in the ED it does not address the issue of translatability. Around one third of patients presenting with concussion to ED in a large UK MTC were aged over 65 (59) therefore such patients should be included in any future analysis. In addition, blood alcohol levels are not routinely taken in the UK and so such results would not be available to any ED clinician assessing potential sufferers of concussion- how would these be interpreted if available in any case?

The pragmatic nature of the CONTACTS study meant that our participants were truer to "real-life" patients presenting to the ED and the conditions in which the assessments would actually be used in the NHS. Overall, the diagnostic utility of both the SCAT5 and ImPACT Quick in the NHS has not been proven and should not be recommended outside of the world of professional sports. Our salivary miRNA results however, are promising.

miRNA

The CONTACTS study has demonstrated three miRNA in the saliva of concussed patients that were dysregulated in the ED; hsa-let-7f, hsa-miR-144-3p and hsa-miR-34b-3p. Hsa-let-7f is a member of the let family which has been demonstrated to choreograph the development of neural progenitors into either neurons or glial cells through regulation of key-protein HMGA2 and NOTCH signalling. (69) Additionally, results from the SCRUM study showed a strong association between let-7 family members and the extracellular (ECM) receptor interactions pathway. (70) ECM disruption has been demonstrated to results in synaptic and neuronal loss in neurodegenerative and psychiatric diseases. (71, 72) Hsa-let-7f itself has found to be downregulated in the blood of patients with terminal Alzheimer's Disease. (73) In paediatric patients it has found to be downregulated in the brain tissue resected from patients with focal cortical dysplasia (74) and upregulated in the serum of patients with the neuropsychiatric Tic disorder. (75) Overall, we can conclude that has-let-7f plays a clear role in the development and maintenance of CNS cells and may extrapolate how these mechanisms also apply following concussion.

A miRNA commonly dysregulated in various solid organ tumours, hsa-miR-144-3p has also been implicated in several neurodegenerative diseases including Alzheimer's Disease, drug-naïve Parkinson's Disease and Huntington's Disease. (76-79) It has also demonstrated its ability to alter the oncological behaviour of glioma cells through the regulation of Transferrin Receptor Protein (TFRC) in patients with glioblastoma. (80) A positive correlation with psychiatric symptom scores has too been identified (81) and hsa-miR-144-3p levels change in the presence of calcitonin gene-related peptide (CGRP) blockers for the treatment of migraine suggesting its role in the regulation of central vasodilation. (82) Of our three dysregulated miRNAs, hsa-miR-34b-3p appears to have the least evidence published in neurological disease although it has been found to be dysregulated in the umbilical cord blood of neonates with perinatal asphyxia. (83)

Of the 14 miRNAs identified in the SCRUM study (70) only three were found to be dysregulated in CONTACTS. The two main factors postulated for this are; 1- the heterogeneity of the CONTACTS population and 2- the timings of the saliva samples taken. Our patient sample had a wide age range (16-68 years), contained male and female subjects and did not exclude patients with premorbid disease. This sample is more representative of our NHS population compared to a group of young, healthy male athletes and contains many more variables that can affect levels of miRNA. Both sex and age have been found to affect miRNA levels and the ageing process itself is known to change miRNA expression. (84, 85) The timing of our sampling was also variable and although within 24 hours of injury, was a far-cry from the uniform pitch-side and post-game tests in SCRUM. However, this is again another example of how our study group is more comparable to the NHS population- arriving at a wide range of times following injury. Despite the heterogenous nature of the CONTACTS sample however, there remains a diagnostic signal in this handful of miRNAs.

It is hypothesised that miRNAs are delivered directly from cranial nerves into the oropharynx, providing a rapid response within saliva after TBI. (86) This is further supported by the ultra-early, pitch side diagnostic value of salivary miRNA in the SCRUM study. (70) As already mentioned, both SCAT5 and ImPACT Quick assessments are not translatable to a busy NHS ED. Saliva sampling however, is quick, non-invasive and, in this study, performed by the patients themselves in most cases. Compared to serum sampling, a saliva test is more readily accepted by patients, an important factor in the development of a future diagnostic test.

How exactly these miRNAs play a role following injury is unknown and further mechanistic studies in concussion patients could identify the exact pathways and genes targeted by these miRNAs. Further analysis of the CONTACTS samples are underway to investigate several small non-coding RNA (sncRNA) markers also identified in the SCRUM study. Hopefully these results will give further insight into the pathophysiology of concussion and add to the diagnostic profile of those miRNA already discovered.

Limitations

This was an exploratory study, and as such was not powered. In addition, although relatively pragmatic according to PRECIS-2 criteria, (87) the CONTACTS study inclusion criteria were too sctrict leading to many patients being ineligible for recruitment. Future concussion studies should be designed around the existing patient pathways to best mirror real-life and increase

the translatability of any results. Following on from this, only including patients with maxillofacial trauma in the concussed cohort, may have skewed results and future studies should include all patients with clinically diagnosed concussion.

Funding for the study limited laboratory analysis to the pre-determined set of miRNA identified in the SCRUM study. (70) Given the heterogeneity of our sample there may be preferential diagnostic markers outside of the assays tested. Future studies should consider the use of next-generation sequencing to identify a wider range of potential biomarkers.

CONCLUSION

Whilst the SCAT5 and ImPACT Quick assessments have proven useless in the identification of concussion in the ED, salivary miRNAs show diagnostic potential. A quick and non-invasive test, saliva sampling is acceptable to patients and reflects rapid miRNA changes following concussion. Future research should continue to include a wide range of patients and be designed around existing patient pathways to make results more readily translatable to real-life NHS Emergency Departments and consider next-generation sequencing to identify a wider range of budding diagnostic biomarker candidates.

CONFLICT OF INTERESTS

Both Prof Belli and Dr Di Pietro are directors of Marker Diagnostics

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