Issue 9

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pathology@viapath

Inside this issue

Message from the Editor

With December comes wintry weather; frost, hail, snow and ice. Oddly, this reminds me of Viapath's values: innovation, collaboration and expertise (ICE!). This edition of "pathology@viapath" shows how these key pillars are put into practice on a daily basis. Many of Viapath's research teams collaborate with other teams, some of them worldwide, and the two articles on external quality assurance schemes demonstrate the fruits of these liaisons and indicate how innovation is improving the quality of test reporting. Neuron specific enolase is becoming a valuable marker in several different disease states and innovative work has brought about the introduction of a new service for this enzyme which will improve the efficiency of patient testing. Innovation and collaboration have been key in the development of another new service which will help to monitor levels of vitamin B_{12} in patients with a vitamin B_{12} deficiency. This disease is often overlooked and it is envisaged that this new service will assist patients in taking control of their own health. Moreover all the articles in this edition showcase Viapath's expertise in pathology; I hope you find them of interest.

Launch of a 24/7 Neuron Specific Enolase Assay

On 1st December 2017, Viapath introduced a new, rapid turnaround time, 24 hour service for Neuron specific enolase (NSE) at St. Thomas' Hospital using the Roche e602 immunoassay platform.

What is NSE?

NSE is a cell-specific isoenzyme of the glycolytic enzyme, enolase. It is found at high concentrations in neural tissue, cells with neuroendocrine function, platelets and erythrocytes.

NSE is a valuable marker of small cell lung carcinoma (SCLC), with elevated levels being found in 60-81% of SCLC. NSE concentrations correlate with tumour burden, number of metastatic sites and response to treatment. Increased levels of NSE have been reported also in non-small cell lung cancer, but its use has not yet been widely accepted.

NSE is also present in malignant tumours with neuroendocrine differentiation, including neuroblastoma, where increased levels are seen in all stages of disease. Approximately 96% of patients with metastatic neuroblastomas demonstrate an elevated NSE level, which has been associated with a poor prognosis.

Elevated NSE levels may also occur in a wide variety of other tumours including melanoma, seminoma, renal cell carcinoma, Merkel cell tumour, carcinoid tumours, dysgerminomas and immature teratomas, and malignant phaeochromocytomas.

Figure 1: Courtesy of The Kids Cancer Project.

Due to its high concentration in neural tissue, elevated NSE is seen in cerebral tissue damage due to head injury or following ischaemic stroke, hypoxic brain injury intracerebral haemorrhage, inflammatory brain diseases and Creutzfeldt-Jakob disease.

KEY MESSAGES	
	NSE is the tumour marker of choice for staging and for monitoring response to therapy in patients with SCLC
	Elevated NSE is associated with metastatic disease and poor prognosis in neuroblastoma
	NSE is used as a marker of neurological outcome following cardiac arrest

NSE as a Marker of Neurological Outcome Following Cardiac Arrest

There is a lot of interest in world of Intensive Care for the use of NSE as a marker of outcome in patients following cardiac arrest. Despite improving resuscitation practices, the outcome of most patients after a cardiac arrest remains poor. Comatose patients admitted to an intensive care unit (ICU) after an out-of-hospital cardiac arrest (OHCA) have a mortality rate of around 50%. In the majority of cases, initial ICU mortality is driven by haemodynamic failure, whereas later on, morbidity and mortality are due to brain damage.

Various studies have been undertaken to assess the utility of NSE as a marker in this setting to predict poor outcome (death, persistent vegetative state or severe neurological disability). Publications which have considered NSE have included professional body publications as well as research studies. The European Resuscitation Council and the European Society of Intensive Care Medicine released a joint expert panel statement in 2014 recommending the measurement of NSE for the prognostication of neurological outcome following cardiac arrest.

KEY MESSAGES	
	OHCA is associated with 50% mortality and the leading cause of death in these cases is severe neurological injury
	High levels of brain biomarkers such as NSE may identify patients prone to poor outcomes after resuscitation from OHCA
	Outcome prediction after cardiac arrest guides the decision on continuation or withdrawal of intensive care

IMPORTANT PUBLICATIONS:

- A paper published on behalf of the American Academy of Neurology, indicated that increased NSE at days 1-3 post CPR accurately predicted poor neurological outcome (Wijdicks et al. 2006).
- Grubb et al. (2007) demonstrated that NSE levels were significantly higher in patients who died following cardiac arrest. In addition, NSE measured at 24-48 hours after OHCA predicted in-hospital death (ROC AUC 0.81).
- Stammet et al. (2015) demonstrated that NSE was a robust predictor of neurological outcome in OHCA patients, with higher NSE levels associated with decreased survival (sensitivity 70% and specificity 90% at 48 h).
- In an international multi-centre study of >1000 cardiac arrest patients, elevated NSE reliably predicted poor neurological outcome at ICU discharge (PPV 99%) with low levels accurately excluding poor outcome with an NPV of 92% (Streitberger et al 2017).

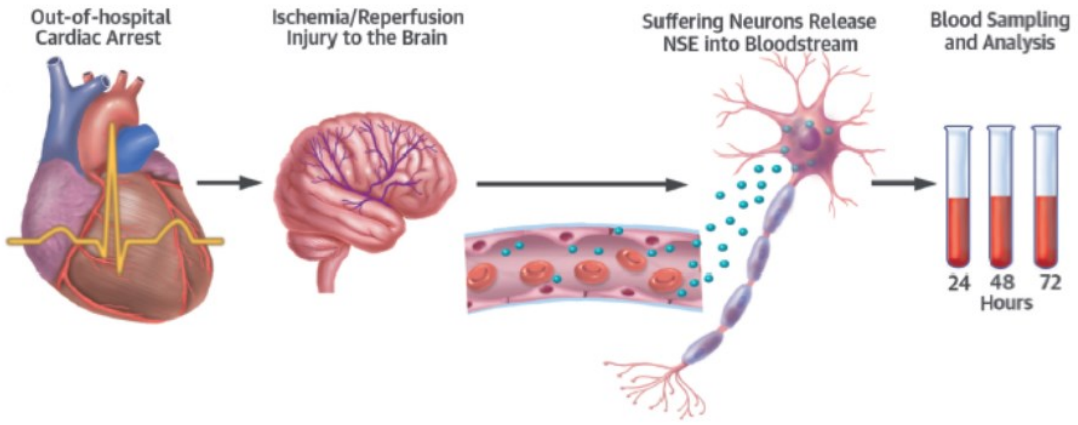

Figure 2: Adapted from Stammet et al. 2015.

Find Out More

Please contact the Blood Sciences laboratory at St. Thomas' for further information on NSE analysis on 0207 188 9247 or by email:

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NASCOLA post-analytical platelet external quality assurance (EQA). More than just an EQA scheme?

How are platelets involved in haemostasis?

After tissue injury an intricate series of events are activated to promote clot formation and prevent blood loss. These events consist of two stages: primary haemostasis, as the name indicates, is the immediate event that occurs following tissue injury, where platelets play a central role in forming a haemostatic platelet plug at the site of injury. This is followed by secondary haemostasis which involves the coagulation factor proteins which work in a cascade to form a blood clot, a more stable means of preventing excessive blood loss.

Normal platelet function is pivotal for the primary haemostasis process as the platelet plug is essential in the control of blood loss. When tissue injury occurs, the endothelial cells on the exposed vessel wall will start to release substances which can activate platelets. The activation of platelets causes them to change shape so that they can adhere to the vessel wall via certain surface receptors. Multiple platelets adhere to the site in this way and, as a result, stick together and aggregate. It is this aggregation that leads to the formation of the essential platelet plug at the site of blood loss. Therefore, platelet function testing is crucial for the diagnostic evaluation of common and rare bleeding disorders.

Background history on EQA consensus guidelines:

Guidelines on platelet function testing that provide information on acceptable methods, including light transmission aggregometry (LTA), were published in 2008 by the Clinical and Laboratory Standards Institute (CLSI). However these guidelines did not address the interpretation of platelet function testing.

In an effort to improve and standardise the evaluation of platelet function disorders, a study involving diagnostic laboratories in North America and the North American Specialized Coagulation Laboratory Association (NASCOLA) generated consensus guidelines that cover the important aspects of platelet function testing. This includes how to interpret and follow up on LTA abnormalities.

The consensus guidelines have the following key points:

- A list of recommended agonists and the concentrations to use for platelet LTA for common and rare platelet function disorders.
- A recommended approach to determine a reference range for the agonists used.
- A recommended approach to interpret platelet LTA results.

Challenges for the laboratory undertaking platelet function testing

1) It takes a considerable amount of time for a Biomedical Scientist to gain competency in performing the platelet light transmission aggregometry (LTA) testing (the measurement of platelet aggregation in response to specific platelet agonists which stimulates aggregation).

2) Platelet function testing is complex, involving the need for fresh blood samples which have to be processed within four hours.

3) Patient testing must be performed in parallel with a healthy control donor sample.

4) Patients and donor controls must complete a questionnaire to identify any interfering drugs which may produce an abnormal trace.

5) Assessment and interpretation of platelet function testing by LTA needs to be completed by looking at all the global tests (including ATP release assays and platelet morphology) alongside the clinical details for the patient. This requires careful scrutiny of the testing traces with a consultant from the Haemophilia Centre at St Thomas' Hospital.

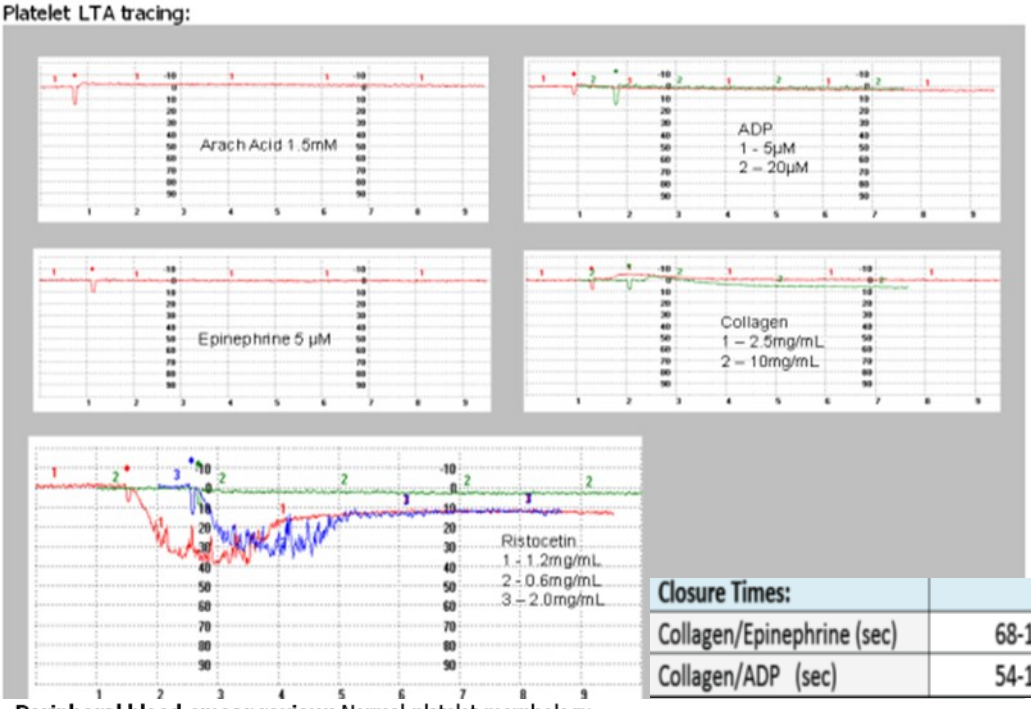
The NASCOLA EQA Scheme

In order to meet these challenges, Viapath's Diagnostic Haemostasis laboratory decided to participate in the NASCOLA EQA scheme which has many benefits for the service user. The NASCOLA EQA scheme is unique: it is a bi-annual post-analytical interpretation exercise with individualised reporting on four case studies which have been developed in collaboration with the NASCOLA platelet working group.

Figure 1: Case study results including LTA traces which shows the level of platelet aggregation in response to the platelet agonists arachidonic acid, ADP, epinephrine, collagen and ristocetin

Collagen (10 mg/mL)	6%	>60%
Ristocetin (0.6 mg/mL)	2%	<10%
Ristocetin (1.2 mg/mL)	35%	>60%
Ristocetin (2.0 mg/mL)	30%	>60%

Platelet LTA tracing:



Closure Times:		
Collagen/Epinephrine (sec)	68-164	237
Collagen/ADP (sec)	54-126	173

Peripheral blood smear review: Normal platelet morphology.

General coagulation testing: Normal PT, APTT and TT. No evidence of VWD. PFA-100 Epinephrine and ADP cartridge closure times are prolonged, >300 seconds and >260 seconds, respectively.

Case study example:

Patient is a 22 month old boy with a history of significant bruising and bleeding since birth. No family history of transfusions, von Willebrand Disease, factor deficiencies or platelet dysfunction.

NASCOLA summary: All platelet aggregation responses to agonists are reduced or absent and PFA is abnormal; the results are indicative of the condition Glanzmann Thrombasthenia (Figure 1).

A panel reviews the reports summary submitted online and return a suggested diagnosis summary plus comments from the reviewer. Participation in the EQA scheme helps to promote collaboration between the laboratory and clinicians to facilitate the process of improving platelet function testing interpretations. NASCOLA has observed a significant improvement in laboratory performance over time on the exercises which suggests post analytical challenges are a helpful addition to laboratory EQA (in addition to fulfil ISO requirements for EQA participation) as well as educational for rarely seen platelet disorders.

For further information on the NASCOLA Post Analytical platelet EQA scheme click here.

The platelet testing service at Viapath

Platelet testing and interpretation is offered at Viapath's Diagnostic Haemostasis & Thrombosis laboratory based at St Thomas' Hospital. For more information about the test and the sample requirements please visit http://www.viapath.co.uk/our-tests/platelet-function-analysis-aggregometry.

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Dr Aine McCormick, Senior Biomedical Scientist
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Reference

Hayward CPM,A Development of North American Consensus Guidelines for Medical Laboratories That Perform and Interpret Platelet Function Testing using light Transmission Aggregometry. Am J Clin Pathol 2010 134:955-963.

Platelet function testing is important for the diagnostic evaluation of common and rare bleeding disorders.

Laboratory Quality Assurance for Rare Specialist Assays

The Importance of External Quality Assessment in a Diagnostic Laboratory

External quality assessment (EQA) is intended to achieve inter-laboratory and inter-instrument harmonisation of results and to monitor the general level of performance in a laboratory. This assessment is retrospective and provides random examples of the way in which the laboratory performs. In a national scheme, a laboratory can check their performance regularly against that of other laboratories throughout the country including other laboratories using similar equipment, reagents and procedures. Participation in EQA schemes can help identify anomalies such as faults in instruments, reagents, preparations of quality control (QC) material or method procedures and help to ensure continuation of good laboratory practice.

Laboratory Quality Assurance for Rare Specialist Assays (LQARSA)

All laboratories need to be assessed against the International Standard ISO 15189:2012, to gain and subsequently retain UKAS accreditation. This is used as the basis to evaluate the competence of laboratories, as well as providing quality assurance for some of the rare specialist assays for which no EQA schemes are currently available. These tests do not have an affiliated EQA scheme, as they are performed in only a few specialised laboratories, often those that are linked to a trauma centre and/or Haemophilia Centre. Examples of such tests include platelet aggregation studies and Platelet Function Assays (PFA) for which samples must be processed and completed within 4 hours of the sample collection time.

In order to comply with the ISO 15189:2012 standards, Senior Biomedical Scientist, Charlotte Noronha implemented the LQARSA scheme in Viapath's Diagnostic Haemostasis & Thrombosis laboratories.

LQARSA test	Sample exchange between Viapath and…
Bethesda assays for inhibitors to clotting factors other than FVIII and FIX. RIPA (Ristocetin platelet induced aggregation) VWF (von Willebrand Factor) inhibitor PFA-100 (Platelet function assay) Anti-factor VIII ELISA HIT-A (Heparin-induced thrombocytopenia aggregometry test)	Royal London Hospital
Platelet Aggregometry Fibrinogen antigen Reptilase Time PFA-100	Basingstoke Hospital
Factor Antigen Platelet Nucleotides	Royal Free Hospital
Platelet Glycoproteins	Great Ormond Street Hospital
ATP release measurement (Chronolog)	Hammersmith Hospital

The scheme involves the exchange of specimens, which have already been tested at Viapath and other major Specialist centres across London and the UK (Table 1). This inter-laboratory sample comparison takes place at least twice a year and is dependent on the availability and suitability of samples, due to the rare nature of the tests.

Each LQARSA sample is analysed and the results compared between each specialist centre. If the results are not in agreement, advice must be sought from both laboratories' scientific lead; there will be joint discussions between both laboratories to reach a mutually acceptable outcome. A time frame of four weeks is given to resolve an issue and if this cannot be achieved, communication regarding a pending investigation must be actioned. At Viapath, decisions to take action must be discussed with Dr Gary Moore, Consultant Biomedical Scientist, to facilitate further investigation. The details of this will also be relayed to the governance report via Quality Control error logs produced with all non-consensus results.

Inaugural Innovation and Collaboration Meeting

The Inaugural Innovation and Collaboration Meeting was held at St Thomas' Hospital on Tuesday 10th October 2017 with Viapath representatives from the Diagnostic Haemostasis & Thrombosis department and colleagues from other specialist hospitals in London and the UK. The purpose of the meeting was to share experiences in regards to sample exchange and comparing ISO non conformances. It was also used as a forum to discuss developments in cross-site testing and proposals for improving sample transit time between sites as sample processing time is critical. Furthermore, the implementation of intra-laboratory quality assessment where no cross-site exchange is possible was discussed; suggestions included re-analysing a sample previously tested and comparing the results to assess reproducibility and accuracy.

Helen Lewis, Haemostasis Diagnostics Manager at Basingstoke hospital, remarked "It was a most valuable opportunity to meet like minded scientists with very specific challenges, it has given us much to consider!"

Kurtis Lee, Lead Biomedical Scientist in the Haemostasis department at Hammersmith Hospital, thought "The meeting was particularly informative and provided a useful insight into various UKAS non-conformances and how these have been closed with different approaches".

The LQARSA scheme involves the exchange of samples and comparison of results between Viapath and other major Specialist centres across London and the UK.

From left to right: ***Parmilla Dhamrait*** *(Medical Laboratory Assistant, Viapath),* ***Helen Lewis*** *(Haemostasis Diagnostic Manager, Basingstoke Hospital),* ***Charlotte Noronha*** *(Senior Biomedical Scientist, Viapath),* ***Clive Burgess*** *(Haematology Laboratory Manager, Great Ormond Street Hospital),* ***Nina Kaur*** *(Quality Manager, Bart's Health NHS Trust),* ***Sean Platton*** *(Principal Biomedical Scientist, Bart's Health NHS Trust),* ***Dr Jane Needham*** *(Consultant Biomedical Scientist, Basingstoke Hospital),* ***Anne Riddell*** *(Laboratory Manager, Health Services Labs, Royal Free),* ***Dr Aine McCormick*** *(Senior Biomedical Scientist, Viapath),* ***Kurtis Lee*** *(Lead Biomedical Scientist, Imperial College NHS Trust),* ***Dr Gary Moore*** *(Consultant Biomedical Scientist, Viapath),* ***Jacky Cutler*** *(Operations Manager, Viapath).*

Future Developments for LQARSA

Expansion of the LQARSA scheme was also discussed to incorporate rare specialist tests such as ATP release measurement (Chronolog), Anti-factor VIII ELISA and HIT-A to the repertoire of tests (Table 1). Furthermore, a proposal to include other specialist centres such as Kent and Canterbury and King's College hospitals was suggested. Due to the success of this meeting, a future meeting is to be scheduled next year.

For further information about the LQARSA scheme, please contact:

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Skewed T Follicular Helper Cell Subsets in Common Variable Immunodeficiency

What is common variable immunodeficiency?

Common variable immunodeficiency (CVID) is one of the most frequent, clinically significant primary immunodeficiencies. It is a heterogeneous collection of disorders characterised by impaired antibody secretion (1-4). As a consequence of the failure to produce protective levels of antibodies, sinopulmonary infections are very common, with approximately 95% of patients suffering with pneumonia, bronchitis or sinusitis (2), and so chronic lung disease and bronchiectasis are frequently encountered complications. Patients with CVID therefore require replacement antibody therapy in order to improve their quality of life, reduce the frequency of infections and enhance their survival (5). However, there are also subsets of CVID patients that present with a range of other clinical features including malignant, inflammatory and autoimmune conditions (6). These associated conditions are not improved by antibody replacement, can be difficult to treat due to the need for immunosuppressive therapies and are now thought to be the main cause of morbidity and mortality (3). Hence there is a need to accurately stratify CVID patients.

The role of T follicular helper cells

In an effective antibody response (Figure 1), antigen specific B cells are stimulated to proliferate in germinal centres, where they undergo affinity maturation and class switching. However, this germinal centre reaction may be defective in some CVID patients who are often found to have decreased levels of class switched memory B cells (smB) (5-8).

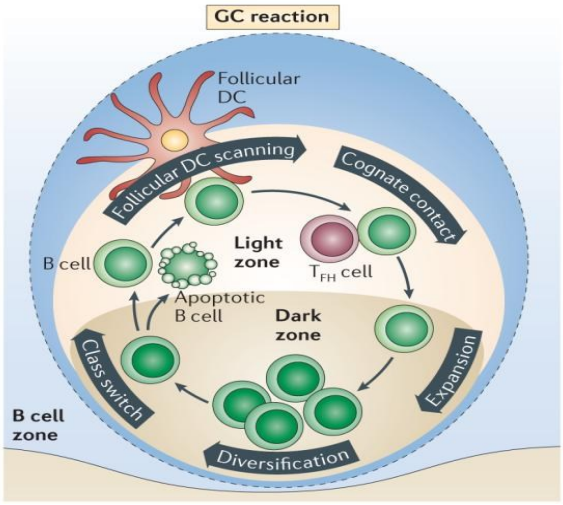

Figure 1: The germinal centre reaction (9).

The importance of T follicular helper cells (Tfh) in the germinal centre is well known (10), where they provide numerous signals to the germinal centre B cells (11). Whilst Tfh are found in the germinal centres by definition, it is recognised that Tfh have a counterpart present in the circulation, meaning whole blood samples can be used to more easily study Tfh (12). Tfh are differentiated from the other T helper cell lineages as they uniquely express chemokine C-X-C motif receptor 5 (CXCR5), which enables migration into the B cell follicle, and they are further subdivided based on the presence of co-expressed CXCR3 and C-C motif chemokine receptor 6 (CCR6) to define three Tfh subsets: Tfh1, Tfh2, and Tfh17 (12,13). Given that the Tfh subsets differentially influence class switching and antibody secretion, the aim of the present study was to determine whether Tfh subset defects were present in CVID (12).

T follicular helper cells and CVID

As the Tfh and their subsets are easily distinguishable by a set of cell surface receptors, flow cytometry is an ideal method to use for their analysis. Circulating Tfh subsets were compared between 31 healthy controls and 24 CVID patients, using a novel whole blood, 9 colour flow cytometric assay and a Beckman Coulter Gallios flow cytometer. The assay also incorporated PD1 and ICOS expression to interrogate the activation status of each Tfh subset (15). Samples were also sent to University Hospital of Wales, Cardiff, for characterisation of B cells.

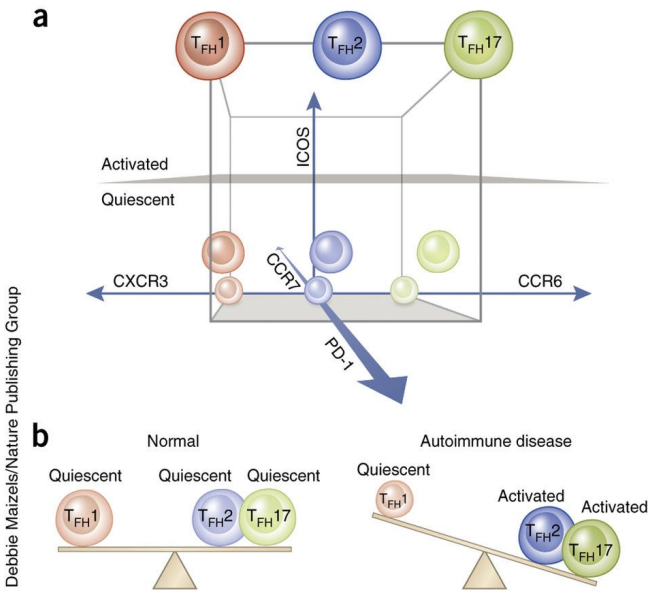

Figure 2: a) subsets of Tfh b) Tfh subsets in health compared to some autoimmune diseases, such as SLE, juvenile dermatomyositis, Sjögren's syndrome or multiple sclerosis (15).

It was hypothesised that skewed Tfh subsets may provide a mechanism to explain the heterogeneity found in CVID patients, given that different Tfh subsets, like T helper cells, are stimulated by different signals received from the microenvironment and secrete different cytokines (11). Furthermore, dysregulation between the subsets has already been noted in several autoimmune conditions (Figure 2), but there is a paucity of data applying Tfh subset analysis to primary immunodeficiency (12).

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At the IBMS Congress, 2017, Charlotte Lee submitted this work as poster and won the Immunology Category Award. The Immunology research group will be publishing their work in the near future and revealing their findings.

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Are you suffering from a Vitamin B_{12} deficiency and need additional help?

Why is Vitamin B_{12} important?

Vitamin B_{12} (B_{12}) is involved in the metabolism of every cell of the human body, especially affecting DNA synthesis, fatty acid and amino acid metabolism. It is required for the formation of red blood cells and in the function of the brain and nervous system.

Why does Vitamin B_{12} deficiency occur?

The human body cannot make B_{12}, so it must be obtained from the diet. Sources of B_{12} include fish, shellfish, meat, liver, eggs, poultry and dairy products. Therefore B_{12} deficiency can occur due to the adherence to vegetarian and vegan diets which restrict the dietary intake of animal products and hence B_{12}.

Patients with Pernicious Anaemia also suffer from B_{12} deficiency. This is due to the absence of intrinsic factor (a glycoprotein). Intrinsic factor is usually present in the gut and is essential for efficient B_{12} absorption. Thus its absence leads to B_{12} deficiency.

It is widely assumed that once that deficiency is corrected the patient will feel well again and the symptoms will disappear. Whilst this happens with some patients, many will still experience the symptoms with various degrees of intensity, whilst others will not experience any lessening of the symptoms and will be faced with having to make some life-changing decisions relating to their work and family life.

Research into patients with B_{12} deficiency

For many years, scientists at Viapath have been studying B_{12} with a particular focus on the laboratory assessment of status. Recently, working with the Pernicious Anaemia Society, it was discovered that many suffers of pernicious anaemia feel underserved and frequently look for supplementary care. This study has led Viapath to design a new service (Viapath Nutris) to support patients with gaining a better understanding of their B_{12} status and the management of a deficient state, by getting direct access to relevant state-of-the-art test panels.

Viapath has also been learning from vegans. Vegans often take a proactive approach to their health but, are aware that they are prone to B12 deficiency, many taking supplements to prevent this. However, the research indicated that many would like to take a more scientific approach to monitoring B12 to ensure their efforts to manage it are effective.

Viapath Nutris

Viapath is introducing a new service that will allow patients to go online, select a test panel, and in some cases, have results within 24 hours of having their blood drawn. Clear, jargon-free information will help users to select the right test for them and understand their results, which include a personalised interpretation and links to scientifically backed, in-depth and cutting-edge articles and information on nutritional science and related pathologies.

Viapath Nutris coming soon.

For Customer Service related issues please contact our dedicated team on:
020 7188 8008 (Viapath at St Thomas' Hospital)
020 3299 3576 (Viapath at King's College Hospital)
customersupport@viapath.co.uk

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