Flow cytometry plays an important role in monitoring the efficacy and duration of B cell depletion after the use of anti-CD20 monoclonal antibodies including rituximab. The sensitivity of this testing is dependent, amongst other things, on the total number of events collected and this can vary between laboratories. Recently, the phenotype of the B cells at the time of repopulation has been investigated as a possible marker to predict disease relapse in the setting of ANCA positive vasculitis. These studies will be discussed. Flow cytometric evaluation of T cell subsets including evaluation of regulatory T cells and T follicular helper cells have also been used in the experimental, rather than diagnostic setting, to evaluate the efficacy of anti-CD20 therapy for induction of disease remission.

Flow cytometry has also been used in the experimental setting to investigate the cellular consequences of treatment with a number of other monoclonal antibodies including abatacept and tocilizumab.

TESTS FOR TTP/aHUS

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Thrombotic thrombocytopenic purpura (TTP) follows an excess of ultra large multimers of von Willebrand factor (vWF), which follows reduced serum ADAMTS13 activity. Microangiopathy which follows intoxication with Shiga toxins, e.g., some *E. coli* (STEC) are perhaps best labelled as STEC-HUS. ADAMTS13 activity and stool STEC toxin are specific assays for these diseases.

Many STEC negative/ADAMTS13 replete cases are complement mediated. Organ damage will occur without affecting routine complement assays. Triggering events are minor and not specific for microangiopathy (e.g., rotavirus). Testing aims to demonstrate (1) the predisposing tendency to complement-mediated microangiopathy (detected in ~30% of cases) or (2) complement activation (largely anecdotal and case studies).

Abnormalities of regulators of complement activation (RCA) molecules have the highest yield, specifically, complement factors H and I (CFH, CFI), and membrane cofactor protein (MCP, CD46). Complement component 3 (C3), complement factor B, thrombomodulin have a lower yield.

Complement activation can be demonstrated with serum levels of terminal complement complex (TCC, aka sMAC, or sC5b-9) and biopsies for vascular deposition of the same TCC/MAC (either involved organ or random skin biopsies).

Reference

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AN UPDATE ON TTP INCLUDING DATA FROM THE AUSTRALIAN TTP/TMA REGISTRY

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Thrombotic thrombocytopenic purpura (TTP) is a life-threatening thrombotic microangiopathy (TMA) which is the result of

a severe deficiency of ADAMTS13 activity. In the majority of cases, the ADAMTS13 deficiency is the result of auto-antibodies produced against ADAMTS13 which impair protein activity. With the recognition and characterisation of the pathological basis of acquired TTP has come rational therapies including more targeted and less toxic immunosuppression (e.g. rituximab), targeted agents against the vWF platelet interaction (caplacizumab) and recombinant ADAMTS13.

The Australian TTP/TMA registry was established to collect data on Australian patients with all TMAs with the aim of creating a resource to help assess the patterns of diagnosis and management of this group of disorders in Australia. The registry is maintained by the Monash University Department of Epidemiology and Preventive Medicine (DEPM) under the auspices of the Transfusion Outcomes Research Collaboration, a partnership with the Australian Red Cross Blood Service (ARCBS), and began collecting data in 2009. Analysis of data from patients with TTP in the TTP/TMA registry has given insights into the current state of diagnosis and treatment of TTP in Australian patients and identified potential areas for practice improvement which will be shared in this presentation.

aHUS IN CHILDREN: A SEVERE DISEASE WITH INCREASINGLY BETTER DIAGNOSTIC AND TREATMENT OPTIONS

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Atypical haemolytic uraemic syndrome (aHUS) accounts for around 10% of children presenting with haemolytic uraemic syndrome, a triad of thrombocytopenia, haemolytic anaemia and renal failure. As opposed to diarrhoeal causes usually associated with *E. coli* infection producing the toxin STEC, aHUS generally presents without diarrhoea and is frequently associated with defects in the complement pathway leading to over-activation of complement.

The major cause of paediatric aHUS is due to defects in complement factor H, a regulatory protein that limits complement activation. Mutations in CFH that are both dominant and recessive have been described. There are now over 10 other genes that have been described in aHUS with around 40% of sporadic cases of aHUS without an identified genetic cause.

Clinically patients often present acutely with an infectious prodrome, and may require supportive haemodialysis and transfusion. Children occasionally have extra renal manifestations involving GI and CNS. Therapy involves plasma exchange to replace complement factors and more recently the use of C5 inhibitors that have been highly effective.

In those who progress to end-stage renal failure, transplantation carries a high risk of disease recurrence and this is highest with CFH mutations.

Exclusion of infectious HUS and of TTP, a similar adult disease due to defects in ADAMTS13, is important. Genetic diagnosis has been significantly aided by massively parallel sequencing that can sequence the 10 known genes in one run. Further analysis using MLPA to identify rearrangements and deletions is also increasingly of use. Genetic identification assists in risk stratification and in identifying those children who will benefit from C5 inhibitors.