EVALUATION OF METHODS TO MITIGATE THE INTERFERENCE CAUSED BY DARATUMUMAB IN PRE-TRANSFUSION SEROLOGICAL TESTING.

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Introduction

Daratumumab (DARA) is an anti-CD 38 human monoclonal antibody licensed for use in relapsed/refractory multiple myeloma. CD38 is highly expressed on myeloma cells and to a lesser extent other haemopoietic cells. DARA binds CD38 on red cells tightly in vitro causing pan-agglutination in the indirect antiglobulin test (IAT). This pan-agglutination can mask red cell alloantibodies capable of causing haemolysis, complicating red cell provision for these patients. Various methods of counteracting DARA interference have been described including dithiothreitol (DTT)/trypsin treatment of red cells, cord cells, adsorption cells and recombinant soluble human CD38 (sCD38) or DARA idiotype antibody.

Aims

Our aim was to establish a feasible method for mitigating DARA interference to allow safer provision of blood for patients receiving DARA. Neither sCD38 nor DARA anti-idiotype antibody are commercially available and therefore not evaluated.

Materials and Methods

Five different methods were evaluated: allo-adsorption studies, DTT treatment of reagent red cells using three different techniques and cord cells as reagent cells. Allo-adsorption studies were performed using various ratios of red cells to plasma, papain treated and untreated cells and the use of LISS versus non-LISS methods. Testing was performed on plasma containing known antibodies spiked with DARA at three different concentrations 1µg/ml, 10µg/ml and 35µg/ml. DTT treated reagent red cells were tested against spiked plasma using direct tube technique, LISS tube technique and gel column agglutination techniques. Each of the DTT methods was assessed for specificity, sensitivity reproducibility and repeatability. In addition, a selection of phenotyped cord cells were recovered and used as an antibody screening panel.

Results

The optimum adsorption technique was found to be the LISS addition method using a ratio of 4:1:1 with untreated cells. This removed DARA spiked plasma (conc. of 1µg/ml) following four adsorptions but not at a concentration of 35µg/ml. DTT treatment of reagent red cells using direct tube, LISS tube and gel column technique were all successful at mitigating DARA interference and allowing for the presence of underlying antibodies to be identified. The LISS tube and gel column methods were more sensitive than the direct tube method. Underlying antibodies could be detected using reagent DTT treated red cells or phenotyped cord cells.

Conclusions

Of the five methods tested, DTT treatment of reagent red cells proved the most robust method for identification of underlying alloantibodies. CD38 is bound to the red cell surface by disulphide bonds.
As DTT dissolves disulphide bonds, the CD38 antigen is removed also removing bound DARA, allowing serological testing. Disadvantages include a) the DTT treatment process is labour intensive taking approximately 4 hours and b) DTT treatment of red cells removes some red cell antigens including Kell system, Lutheran, Dombrock and YT antigens (and therefore antibodies to these antigens will not be detected using this method). Cord cells lack CD38 expression, allowing for detection of underlying antibodies. This process is quicker than DTT treatment, however, phenotyped reagent cord red cells are not readily available.

References
