SPECIFICATION SPN204/1.1

Diagnosis and Management of T Antigen Activation

This Specification replaces SPN/DDR/RC/024/03 & SPN204/1	**Copy Number**
	Effective **02/11/11**

Summary of Significant Changes

Update to new document code

Purpose

To ensure that a uniform RCI Clinical Policy on the diagnosis and management of T antigen activation is implemented throughout the NBS.

Definitions

None.

Applicable Documents

References

Background

Polyagglutination describes a group of conditions characterised by alterations in red blood cell (RBC) membrane glycoprotein structure in certain pathological states, resulting in the in vitro agglutination of the RBC in the presence of most ABO compatible adult sera.

Classical T antigen activation (referred to as $T_{classical}$ in this document) was the earliest defined form of polyagglutination (Friedenreich 1930). It is caused by cleavage of the neuraminic acid residues from the normally disialylated tetrasaccharides of the MN, Ss and other RBC membrane sialoglycoproteins by the enzymatic action of bacterial neuraminidase. This exposes the hidden beta-linked galactosyl residue (Gal-b(1–3)-GalNAc), the $T_{classical}$ antigen. The unmasked $T_{classical}$ antigen binds with the anti-T IgM antibodies that are normally present in almost all human adult plasma, resulting in agglutination *in vitro* and possible haemolysis *in vivo*.
Different bacterial enzymes may cleave at different sites leading to in vitro agglutination but the clinical sequelae may be different or less well defined than those of classical T activation ($T_{classical}$). Examples include Th, Tk, Tx and Tn. The mechanism of formation of all the variants of T activation and their clinical relevance are documented in table 1. Table 2 documents the action of lectins, polybrene and papain on cells expressing such cryptantigens.

Clinical aspects

There is little evidence that classical T activation causes a significant problems in adults (Adams et al 1989; Buskila *et al*, 1987; Lenz *et al*, 1987; Moores *et al,* 1975: Judd *et al*, 1982: Hubl *et al*, 1993; Rawlinson & Stratton, 1984). There are therefore only 2 clinical scenarios in which classical T activation and related disorders are of clinical relevance in transfusion medicine:

1. Necrotising enterocolitis

Classical T activation is reported in 11–27% of infants with Necrotising enterocolitis (NEC) and in neonates with bowel-related surgical problems (other than NEC) and sepsis (Klein *et al*, 1986; Williams et al 1989; Novak, 1990; Osborn *et al*, 1999; Grant et al 1998). A report on a large series of infants showed no benefit in survival following the introduction of screening and the use of low titre anti-T plasma when compared with unscreened historical controls (Osborn *et al,* 1999). A recent study screened 379 infants for T activation (Boralessa et al 2002). Forty seven had a variant of T activation and only one had classical T activation ($T_{classical}$). 22/48 received transfusion but none displayed evidence of haemolysis. In the same study, 300 random donor samples were screened for the presence of agglutinins, all had anti-T but none had antibodies against the cells with variants of T activation.

2. Atypical Haemolytic Uraemic Syndrome (aHUS)

Neuraminidase producing, invasive *Streptococcus pneumoniae* infection has been recognised as an aetiological factor in atypical haemolytic uraemic syndrome (HUS) in children. This form of HUS is not associated with diarrhoea or the presence of verotoxin. It is sporadic and usually occurs in children under 2 years of age. The T cryptantigen may be exposed in renal endothelium, red cells and platelets. It has been hypothesised as causing the characteristic triad of renal failure, microangiopathic haemolytic anaemia and thrombocytopenia (Klein *et al*, 1977: McGraw *et al*, 1989; Erickson *et al*, 1994 Cabrera *et al*, 1998; McTaggart & Burke, 1998; Shirey et al 1999; Crookston et al 2000).

Guidelines for the investigation of T activation

1) Investigation for T activation should be undertaken in at risk neonates in whom haemolysis has occurred and children with atypical HUS associated with streptococcal infection. Neonates with NEC and other forms of sepsis associated with clostridia, gram negative organisms and streptococcus pneumoniae should be considered at risk.

2) Polyagglutination would not be detected during blood grouping in most laboratories as monoclonal agents are used. If it is encountered full screening for T activation should be undertaken.

3) There is no evidence to support routine screening for T activation in Special Care Baby Unit patients.

4) Not all cases of T activation, even $T_{classical}$, are associated with significant haemolysis, screening should only be undertaken in patients at risk of T activation who have developed haemolysis following transfusion.

5) Screening of patients should be performed at a National Blood Service Red Cell Immunology laboratory before special blood products which have low levels of anti-$T_{classical}$ are provided. Testing should include:

   a) A lectin detecting all forms of T activation (ie Arachis hypogaea)
   b) A method of differentiating classical T activation ($T_{classical}$) from the relatively more prevalent and benign variants such as Tk activation. Glycine soja should be used for all samples that agglutinate with Arachis hypogaea (see Table II, p6).
   c) A Direct Antiglobulin test.
   d) Blood film, reticulocyte count, serum bilirubin, urine and plasma haemoglobin.

6) Patients with variants other than classical T activation, such as Tk activation do not require special blood products.

7) If a neonate (or child with atypical HUS) is at high risk of developing classical T activation but does not agglutinate with Glycine soja repeat testing should be performed if clinical suspicion is raised. The addition of a third lectin that is able to differentiate Th activation from other variants would theoretically be useful (e.g.. Vicia cretica or Medicago disciformis).

Blood products likely to contain significant amounts of IgM anti-$T_{classical}$

i) Fresh frozen plasma
ii) Solvent detergent treated pooled plasma
iii) Pooled and apheresis platelets
iv) Cryoprecipitate
v) Whole blood

Blood products and haemostatic agents that have low levels of IgM complement fixing anti-$T_{classical}$.

i) Red cells in additive solution for neonates and infants
ii) Washed red cells
iii) Platelets in platelet suspension medium (PSM).
iv) Low titre anti-T FFP (The definition of low titre is poorly defined)

v) All fractionated blood products (e.g. coagulation factor concentrates such as II/VII/IX/X concentrate, albumin and intravenous immunoglobulin)

vi) Recombinant factor concentrates (e.g. rVIIa, NovoSeven, FVIII, recombinate / Kogenate, FIX Recombinine).

Provision of blood products for patients with classical T activation (not variants such as Tk, see BCSH 2004)

1) Adults.
Adults do not require special blood components as they have preformed anti-T.

2) Children with atypical HUS.
There is no evidence that donor derived anti-T is detrimental (Massey et al 2003). Virally inactivated fresh frozen plasma may be used if indicated. Platelets are relatively contraindicated (BCSH 2004). Most children with atypical HUS will be older than four months and, like adults, have preformed anti-T. There is no published evidence to suggest that the infusion of FFP has caused worsening of the condition in such children. A small case series had been presented in abstract showing that the infusion of plasma did not lead to a worsening of the condition of affected children (Massey et al 2003). Low titre anti-T plasma is therefore not indicated and standard virally inactivated plasma may be used. BCSH guidelines suggest that platelet transfusions may lead to an acute deterioration in patients with microangiopathies such as HUS and thrombotic thrombocytopenic purpura (TTP). Platelets should therefore only be transfused if the risk of bleeding outweighs the potential risk of worsened thrombotic microangiopathy (BCSH 2004).

3) Neonates who have not had haemolysis but have been screened (contrary to this guidance) for anti-T and found positive.
Provide standard components. There is no evidence that screening and avoiding donor blood components is beneficial as the delays in availability outweigh the benefits (Osborn et al 1999).

4) Neonates who have developed haemolysis following blood component transfusion.

4.1) Efforts should be made to prevent coagulopathy in neonates at high risk of classical T activation (i.e. those with NEC and other forms of sepsis associated with clostridia, gram negative organisms and streptococcus pneumoniae including atypical HUS). Optimal nutritional support should include parenteral vitamin K. If vitamin K is given intravenously repeat doses will be needed if the illness is protracted.

4.2) The need for blood products should as always be carefully considered and the provision of special blood products should involve close liaison between an experienced neonatologist or paediatric nephrologist (aHUS) and haematologist.

4.3) Red cells: Red cells suspended in additive solution (SAG-M) for neonates and infants should be used. Some clinicians have advocated the use of washed red cells for large volume and exchange transfusion but this can lead to considerable delay. There is no evidence to suggest that washed red cells confer any advantage in this situation.

4.4) Platelets: Platelets in platelet suspension medium (PSM) should be used

4.5) Coagulation factors: If the coagulopathy is due to Vitamin K deficiency, vitamin K alone may be effective. For rapid correction of vitamin K deficiency, a combined II/VII/IX/X concentrate may be useful if rapidly available.

4.6) Low titre anti-T FFP. This should be provided if FFP is required to correct a coagulopathy. Donor samples should be screened at a dilution of 1 in 4 by saline RT agglutination against neuraminidase treated red cells. Screen negative samples tested

by saline agglutination at 37^{o}C and found non-reactive should be considered low titre anti-T FFP for recruitment of donors to supply low anti-T FFP. UK sourced single donor plasma virally inactivated with methylene blue may be provided under concession in this situation.

4.7) Both FFP and II/VII/IX/X concentrate in this situation, have shortlived effects (less that 2 hours for some coagulation factors). These blood products should be used in conjunction with parenteral vitamin K.

4.8) Volume expansion: Crystalloid, gelatin, hydroxyethylstarch or human albumin solution.

4.9) Weekly screening for T activation should be performed. There is no need to continue to avoid anti-$T_{classical}$ containing products when $T_{classical}$ is no longer detectable.

Table I. Variants of T activation

Variant	Mechanism	Clinical significance
$T_{classical}$	Cleavage of neuraminic acid residues by bacterial neuraminidases	Causes in vitro polyagglutination and may in some cases cause in vivo haemolysis. Increased incidence associated with Necrotising enterocolitis and sepsis in neonates.
Th	An incomplete or intermediate form of T activation.	Further action by stronger neuraminidases results in the classic T activation
Tk	The result of microbial β-galactosidases that cleave a galactose residue from paragloboside, exposing *N*-acetylglucosamine, the Tk receptor.	One case of Tk activation and massive haemolysis has been reported. Many reports and studies have not differentiated T from Tk activation. Antibodies to such variants are rarely found in donor plasma and low titre products are therefore not required
Tx	The molecular mechanism of this condition has not been clarified (Wolach *et al*, 1987).	Transient Tx activation has been described in children with pneumococcal infection and also in a child with acute haemolytic anaemia.
Tn	Generally the result of mutation in a clone of red cells, with the abnormal cells containing a cryptantigen with only one acetylated galactose residue without beta-linkage, secondary to the absence of the enzyme β-3-galactosyltransferase (β3GT) (Cartron et al 1978).Transient Tn in neonates, associated with a maternal antenatal flu–like illness, probably results from a temporary fetal deficiency of β3GT. (Rose et al 1983)	Tn polyagglutination has been associated with myelodysplastic syndromes and acute leukaemia in adults. The significance of transient Tn in neonates is unclear.

Table II. Lectin panel classification of T-polyagglutination variants (Horn 1999, Ramasethu & Luban 2001).

Lectin / reagent	**T**	**Th**	**Tk**	**Tx**	**Tn**
*Arachis hypogaea**	+	+	+	+	–
*Glycine soja**	+	–	–	–	+
Vicia cretica	+	+	–	–	–
Medicago disciformis	+	+	–	–	–
*Salvia sclarea**	–	–	–	–	+
*Salvia horminum**	–	–	–	–	+
Bandeiraea simplicifolia II	–	–	+	–	–
Vicia hyrcanica	+	+	+		–
Cord Serum	-	-	-	-	-
Polybrene	-		+	-	-
Papain & A hypogaea	+	-	++		-

* lectin is a component of the Gamma Lectin System (Gamma Biologicals Inc.)
+ denotes agglutination occurs
++ denotes increased agglutination compared with untreated cells

References

Adams, M., Toy, P.T. & Reid, M.E. (1989) Exposure of cryptantigens on red cell membranes in patients with acquired immunodeficiency syndrome or AIDS- related complex. *Journal of Acquired Immune Deficiency Syndromes*, **2**, 224–228.

BCSH 2003. Guidelines on the management of the thrombotic microangiopathic haemolytic anaemias. *British Journal of Haematology*, **120 (4)**, 556-573.

BCSH 2004. Transfusion Guidelines for neonates and older children. *British Journal of Haematology*, **124**, 433-453.

Boralessa, H., Modi, N., Cockburn, H., Malde, R., Edwards, M., Roberts, I., & Letsky, E (2002). RBC T activation and hemolysis in a neonatal intensive care population: implications for transfusion practice. *Transfusion* **42** (11), 1428-34

Buskila, D., Levene, C., Bird, G.W.G. & Levene, N.A. (1987) Polyagglutination in hospitalised patients: a prospective study. *Vox Sanguinis*, **52**, 99.–102.

Cabrera, G.R., Fortenberry, J.D., Warshaw, B.L., Chambliss, C.R., Butler, J.C. & Cooperstone, B.G. (1998) Hemolytic uremic syndrome associated with invasive *Streptococcus pneumoniae* infection. *Pediatrics*, **101**, 699–703.

Cartron, J.P., Cartron, J., Andreu, G., Salmon, C. & Bird, G.W.G. (1978) Selective deficiency of 3-b-D-galactosyltransferase (T-transferase) in Tn-polyagglutinable erythrocytes. *Lancet*, **i**, 856–857.

Crookston, K.P., Reiner, A.P., Cooper, L.J.N., Sacher, R.A., Blajchman, M.A., Heddle, N.M. (2000) RBC T activation and hemolysis: implications for pediatric transfusion management. *Transfusion*, **40**, 801–812.

Durocher, J.R., Payne, R.C. & Conrad, M.E. (1975) Role of sialic acid in erythrocyte survival. *Blood*, **45**, 11–20.

Eder, A.F. & Manno C.S. (2001) Does red-cell T activation matter? *British Journal of Haematology* **114** (1), 25-30

Engelfriet, C.P., Reesink, H.W., Strauss, R.D., Luban, N.L.C., Letsky, E., Modi, N., Zupanska, B., van Leeuwan, E.F., Martin-Vega, C. & Krusius, T. (1999) Blood transfusion in premature or young infants with polyagglutination and activation of the T antigen. *Vox Sanguinis*, **76**, 128–132.

Erickson, L.C., Smith, W.S., Biswas, A.K., Camarca, M.A. & Waecker, Jr, N.J. (1994) Streptococcus pneumoniae-induced hemolytic uremic syndrome: a case for early diagnosis. *Pediatric Nephrology*, **8**, 211–213.

Friedenreich, V. (1930) The Thomsen Hemagglutination Phenomenon. Production of a Specific Receptor Quality in Red Corpuscles by Bacterial Activity. Levin and Munksgaard, Copenhagen.

Grant, H.W., Hadley, G.P., Adhikari, M. & Fernandes-Costa, F. (1998) T-cryptantigen (TCA) activation in surgical neonates: a hidden problem. *Pediatric Surgery International*, **14**, 204–207.

Horn, K.D. (1999) The classification, recognition and significance of polyagglutination in transfusion medicine. *Blood Reviews*, **1999**, 36–44.

Hubl, W., Mostbeck, B., Hartleb, H., Pointner, H., Kofler, K. & Bayer, P.M. (1993) Investigation of the pathogenesis of massive hemolysis in a case of *Clostridium perfringens* septicemia. *Annals of Hematology*, **67**, 145–147

Hysell, J.K., Hysell, J.W., Nichols, M.E., Leonardi, R.G. & Marsh, W.L. (1976) *In vivo* and *in vitro* activation of T-antigen receptors on leukocytes and platelets. *Vox Sanguinis*, **31** (Suppl. 1), 9–15.

Judd, W.J., Oberman, H.A. & Flynn, S. (1982) Fatal intravascular hemolysis associated with T-polyagglutination. *Transfusion*, **22**, 345.

Kirsten, G.F., Smith, J., Pieper, C., Bird, A., Wessels, G., Riphagen, S. & Moore, S. (1996) The necessity for T-cryptantigen activation screening in babies with necrotizing enterocolitis. *South African Medical Journal*, **86**, 546–548.

Klein, P.J., Bulla, M., Newman R.A., Muller, P., Uhlenbruck, G., Schaefer, H.E., Kruger, G. & Fisher, R. (1977) Thomsen- Friedenreich antigen in haemolytic–uremic syndrome. *Lancet*, **i**, 1024–1025.

Klein, R.L., Novak, R.W. & Novak, P.E. (1986) T-cryptantigen exposure in neonatal necrotizing enterocolitis. *Journal of Pediatric Surgery*, **21**, 1155–1158.

Lenz, G., Goes, U., Baron, D., Sugg, U. & Heller, W. (1987) Red blood cell T-activation and hemolysis in surgical intensive care patients with severe infections. *Blut*, **54**, 89–96.

Massey, E., Streetly, M., Win, N., Boralessa, H., Williams, M., Fleetwood, P., Bishop, D., Knight, R., McGraw, M., Mannage, S. & Taylor, M. (2003) Transfusion management and outcomes of patients with neonatal sepsis or atypical haemolytic anaemic syndrome and T activition. *Transfusion Medicine*, **13 (S1)**, p34.

McGraw, M.E., Lenden, M., Stevens, R.F., Postlethwaite, R.J. & Taylor, C.M. (1989) Haemolytic uremic syndrome and the Thomsen Friedenreich antigen. *Pediatric Nephrology*, **3**, 135–139.

McTaggart, S.J. & Burke, J.R. (1998) *Streptococcus pneumoniae*-induced haemolytic uraemic syndrome. *Journal of Paediatrics and Child Health*, **34**, 192–195.

Moores, P., Pudifin, D. & Patel, P.L. (1975) Severe hemolytic anemia in an adult associated with anti-T. *Transfusion*, **15**, 329–333.

Novak, R.W. (1990) The pathobiology of red cell cryptantigen exposure. *Pediatric Pathology*, **10**, 867–875.

Peter, C.S., Feuerhahn, M., Bornhorst, B., Schlaud, M., Ziesing, S., von der Hardt, H. & Poets, C.F. (1999) Necrotizing enterocolitis: is there a relationship to specific pathogens. *European Journal of Pediatrics*, **158**, 67–70.

Obeid, D., Bird, G.W. & Wingham, J. (1977) Prolonged erythrocyte T-polyagglutination in two children with bowel disorders. *Journal of Clinical Pathology*, **30**, 953–955.

Osborn, D.A., Lui, K., Pussell, P., Jana, A.K., Desai, A.S. & Cole, M. (1999) T and Tk antigen activation in necrotizing enterocolitis: manifestations, severity of illness, and effectiveness of testing. *Archives of Disease in Childhood Fetal and Neonatal Edition*, **80**, F192–F197.

Ramasethu, J. & Luban, N.L.C. (2001). T activation. *British Journal of Haematology* **112** (2), 259-263

Rawlinson, V.I. & Stratton, F. (1984) Incidence of T-activation in a hospital population. *Vox Sanguinis*, **46**, 306–317.

Rodwell, R. & Tudehope, D.I. (1993) Screening for cryptantigen exposure and polyagglutination in neonates with suspected necrotizing enterocolitis. *Journal of Paediatrics and Child Health*, **29**, 16–18.

Rose, R.R., Skradski, K.J., Polesky, H.F., Beck, M.L., Judd, W.J. & Rogers, E. (1983) Transient neonatal Tn-activation: another example. *Transfusion*, **23**, 422.

Shirey, R.S., Boyd, J.S., Kronz, J.D., Ness, P.M. & King, K.E. (1999) Polyagglutination: clinician be aware. *Transfusion*, **39**, 36S.

Stoll, B.J. (1994) Epidemiology of necrotizing enterocolitis. *Clinics in Perinatology*, **21**, 205–218.

Voak, D., Cann, R., Finney, D., Fraser, I.D., Mitchell, R., Murphy, M.F Napier, J.A.F., Phillips, P.,

Rejman, A.J., Waters, A.H., Wood, J.K. & the British Committee for Standards in Haematology, Blood Transfusion Task Force (1994) Guidelines for administration of blood products: transfusion of infants and neonates. *Transfusion Medicine*, **4**, 63–69.

Williams, R.A., Brown, E.F., Hurst, D. & Franklin, L.C. (1989) Transfusion of infants with activation of erythrocyte T antigen. *Journal of Pediatrics*, **115**, 949–953.

Wolach, B., Sadan, N., Bird, G.W., Moulds, J.J., Bar-Shany, S., Ben-Porath, D., Levene, N.A., Sela, R. & Levene, C. (1987) Tx polyagglutination in three members of one family. *Acta Haematalogica*, **78**, 45–47

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