

# T and Tk antigen activation in necrotising enterocolitis: manifestations, severity of illness, and effectiveness of testing

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## Abstract

**Aims**—To determine if T or Tk antigen activation is associated with different and more severe manifestations of illness in infants with necrotising enterocolitis (NEC); and if a policy of testing infants with suspected sepsis or NEC for T and Tk antigen activation is effective.

**Methods**—A case-control study of infants with confirmed NEC, born after the introduction of screening, was undertaken: 17 activated infants were compared with 28 non-activated controls, matched for gestation and weight. A historical control study compared the outcome of infants before and after the introduction of testing.

**Results**—Of 201 infants with confirmed NEC, 27 were T or Tk antigen activated—10 (9%) before and 17 (19%) after the introduction of testing. T or Tk antigen activated infants had a significantly higher mortality (35% vs 7%); more frequent (71% vs 21%) and severe haemolysis, hyperkalaemia, renal impairment, acidosis; and they received more colloid for resuscitation. While only known activated infants in both time periods were managed with the use of low titre T antibody blood products, there was a significant increase in mortality (odds ratios 2.6; 95% CI 1.2, 5.6) and incidence of surgery (OR 2.7; 1.5, 4.9) after the introduction of testing. The increased mortality (OR 2.6; 0.8, 5.2) and incidence of surgery (OR 1.8; 0.9, 3.7) were no longer significant after adjustment for several perinatal risk factors.

**Conclusions**—In a retrospective case-control study, routine testing of at risk infants increased the detection rate of T and Tk antigen activation. The use of low titre T plasma products in these patients did not reduce mortality compared with historical controls. A randomised controlled trial of testing in at risk infants, or of the use of low titre T plasma products in babies with NEC and T activation, is warranted.

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**Keywords:** necrotising enterocolitis; polyagglutination; T antigen; Tk antigen

Haemolysis due to polyagglutination of red cells is a life threatening complication of blood product transfusion in sick premature infants.

Haemolysis after transfusion of blood products in infants was first reported by van Loghem in 1955.<sup>1</sup> Many other reports have since been published,<sup>2-10</sup> with most infants having necrotising enterocolitis (NEC). Polyagglutination is the agglutination of red cells by most normal adult sera; it occurs as the result of exposure of antigens (including T and Tk antigens) on the surface of damaged red cells. T antigen activation results from the exposure of red cells to bacterial or viral enzymes.<sup>11</sup> Bacteria<sup>2-6</sup> produce neuraminidase that cleaves terminal N-acetylneuraminic acid residues from the red cell membrane, leaving the T antigen exposed. Antibodies to the T antigen are not usually present in newborn sera, but are found in most adult sera.<sup>12</sup> Springer and Tegtmeyer<sup>13</sup> found that many bacteria and vaccines contain a substance identical to the T antigen which explains why most adults have anti-T antibodies despite not having been exposed to blood products. It is thought that haemolysis from T antigen activation occurs not by anti-T formed by the infant, but by passively transfused anti-T in plasma given to a sick infant.<sup>7</sup> The prevalence of T antigen activation in studies of neonates with NEC has varied from 11 to 27%.<sup>7 14</sup>

Tk activation is due to the exposure of a subterminal N-acetyl glucosamine by a bacterial enzyme N-acetyl-galactosidase that cleaves the terminal galactose residue from the red cell.<sup>11 15-17</sup> How Tk antigen is activated in NEC is not fully understood. To differentiate Tk from T antigen activation requires additional lectin testing of the red cells. In previous reports T and Tk antigen activation may not have been differentiated. Only one fatal case of Tk antigen activation and massive hemolysis in NEC has been reported.<sup>18</sup>

The management of T and Tk antigen activated infants varies. Approaches have included routine testing for T antigen activation of all infants with suspected NEC or sepsis,<sup>9 13</sup> which, in a historical cohort improved the outcome of infants with confirmed NEC.<sup>13</sup> Other measures, such as avoidance of all plasma products in infants with suspected sepsis or NEC,<sup>9</sup> or the use of plasma free products where possible and low titre T antibody products where necessary in activated infants,<sup>13 19</sup> have also been recommended.

In this study we review our experience of T and Tk antigen activation in infants with NEC over a period of 11 years. We subsequently hypothesised that both T and Tk antigen activation in infants with NEC is associated with an increased severity of disease in infants with

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NEC. To address this hypothesis, we performed a case-control study of infants with NEC which compared T and Tk antigen activated infants with non-activated infants.

The impact of routine testing for T and Tk antigen activation in all infants with suspected NEC or sepsis since September 1991 was examined. It was hypothesised that routine testing and avoidance of plasma containing products or use of low titre T products in activated infants would result in a decreased need for surgery and mortality from NEC.

### Methods

Westmead Hospital is a perinatal referral centre for the Western Sydney Area Health Region. During the 11 year study period, the neonatal intensive care unit increased in size from eight ventilator beds to 12 in 1992, without attendant changes in admission policy regarding gestation or birthweight limits. From 1986 to 1996, 5400 newborn infants were admitted to the unit. A retrospective study was conducted using the inhouse computer database, and by medical record and flow chart extraction. A total of 201 infants were diagnosed as having confirmed NEC, defined as radiological evidence of pneumatosis or perforation, or pathologically confirmed NEC during surgery or postmortem examination. Two hundred and thirty seven infants were classified as having clinically suspected NEC. These infants could have had other conditions, such as sepsis or feed intolerance, and so were excluded from this study.

From January 1986 to September 1991, testing for T and Tk antigen activation was performed mainly at the request of the attending neonatologist, usually in infants with NEC who required blood products. Since September 1991, infants with suspected NEC (any illness associated with abdominal signs resulting in the infant being put under a nil by mouth regimen and given parenteral antibiotics) or suspected sepsis (resulting in the use of parenteral antibiotics) have been routinely tested.

NEC infants are managed with respiratory support, cessation of feeds, and intravenous fluids and antibiotics (ampicillin, amikacin, and metronidazole). For hyperkalaemia with serum potassium above 7 mmol/l, an insulin and dextrose infusion was considered. Insulin and dextrose infusions used in our unit have been described before.<sup>20</sup> There was no change in senior surgical staff or surgical management. Greater use of antenatal steroids by obstetric staff from 1991 resulted in increased antenatal steroid exposure from 19% to 72% in very low birthweight infants admitted into the unit.

A panel of lectins (Gamma Biologicals Inc., Houston, Texas) is used to test infants with suspected NEC or sepsis, with Tk antigen activated infants reacting with only *Arachis hypogea*, and T antigen activated infants reacting with *Glycine soja* and *Arachis hypogea*. Since 1986, all infants with known T or Tk antigen activation have been transfused using plasma free products or low titre T blood products, such as 5% albumin, low titre T antibody packed red cells or triple washed red cells,

low titre T antibody platelets, and low titre T antibody fresh frozen plasma (FFP) where necessary. It was not routine practice to use plasma free products or low titre T blood products in infants with no T antigens, although this approach had been used for some infants.

### COMPARISON OF T AND Tk ANTIGEN ACTIVATED INFANTS

Manifestations of illness compared, included need for surgery and mortality from NEC, evidence of haemolysis (presence of fragments and spherocytes or 2+ or more spherocytes on the blood film), hyperbilirubinaemia during the episode of NEC, use of double volume exchange transfusion, and abnormal haematological parameters (thrombocytopenia—platelet count  $< 150 \times 10^9/l$ —need for platelet transfusion, and leucopenia—white cell count  $< 5.0 \times 10^9/l$ ). Haematuria was defined as macroscopic haematuria  $\geq 3+$  or haematuria on urinary Dipstix testing. Haematuria was not distinguished from haemoglobinuria. Evidence of abnormal renal function during the 10 days of NEC included periods of anuria and renal failure, defined by a creatinine  $\geq 120$  mol/l; hyperkalaemia defined as a potassium of 6.5 mmol/l, and the use of insulin and dextrose infusion. Other illness severity indices included hypotension treated with inotropes, and volume and type of colloid and red cells transfused during the 10 days since the start of the episode of NEC.

### CASE-CONTROL STUDY OF ACTIVATED AND NON-ACTIVATED INFANTS

A case-control study was conducted to compare the clinical manifestations of NEC infants with T and Tk antigen activation and those who were not activated since the introduction of universal testing in September 1991. Seventeen NEC infants were T or Tk antigen activated. Controls were selected from the remaining 74 infants on a 2 to 1 basis using an algorithm that included birthweight within 100 g of the case, gestation within 1 week of the case, and nearest possible birth date. If a control was not obtained at first attempt then infants with a gestation within 2 and then 3 weeks were included. Antenatal and postnatal characteristics were compared. Major outcomes included the need for surgical intervention and mortality of the NEC episode. Severity of illness using the above mentioned parameters was also compared.

### HISTORICAL CONTROL STUDY OF THE EFFECT OF TESTING

All infants with confirmed NEC diagnosed before September 1991 were compared with all infants with confirmed NEC diagnosed subsequently. Major outcomes included prevalence of surgery and mortality from NEC. Adjustment for perinatal risk was performed using logistic regression. The explanatory variable was the performance of routine testing of at risk infants for T and Tk antigen activation (born before or after September 1991). Outcome variables were either death or need for surgery. Confounders

Table 1 Perinatal risk factors of infants in case control comparison of 17 T or Tk antigen activated infants and 28 weight and gestation matched controls who developed NEC after September 1991

Variable	T/Tk antigen activated infants (%)	Matched control infants (%)	p Value
Number of infants	17	28	
Hypertensive disease of pregnancy	4 (24%)	5 (18%)	0.7 NS
Antepartum haemorrhage	5 (29%)	8 (29%)	1.0 NS
Growth restriction	1 (6%)	0 (0%)	0.3 NS
Poor flows	5 (29%)	3 (11%)	0.2 NS
Antenatal steroids	12 (71%)	21 (75%)	0.7 NS
Birthweight (g) (SD)	1170 (343)	1137 (332)	0.8 NS
Gestation (weeks) (SD)	28.7 (2.5)	28.5 (2.0)	0.8 NS
Male gender	12 (71%)	13 (46%)	0.1 NS
Apgar score at 5 minutes (SD)	7.1 (2.6)	7.7 (1.3)	0.3 NS
Patent ductus	4 (24%)	9 (32%)	0.7 NS
Indomethacin given	2 (12%)	8 (29%)	0.3 NS
Hyaline membrane disease	6 (35%)	16 (57%)	0.2 NS
Fed before NEC	17 (100%)	26 (93%)	0.5 NS
Only fed breast milk before NEC	5 (29%)	8 (29%)	0.5 NS
Received plasma products before NEC	14 (82%)	27 (96%)	0.1 NS

adjusted for included antepartum haemorrhage, hypertensive disease of pregnancy, fetal growth restriction (<10th percentile), use of antenatal steroids, Apgar score at 5 minutes, birthweight, gestational age, patent ductus arteriosus and hyaline membrane disease.

Fisher's exact test for comparison of categorical variables with the approximation of Woolf for calculation of 95% confidence intervals (INSTAT software, Texas, USA), and Student's *t* test (MINITAB software, Pennsylvania, USA) were used for analysis where appropriate. Logistic regression was performed with SPSS (SPSS version 7.5, Illinois, USA).

### Results

Two hundred and one infants admitted to Westmead Hospital neonatal intensive care had confirmed NEC. The overall mortality of infants with confirmed NEC was 16.9%, with surgery performed in 43%. Twenty seven infants were identified as T or Tk antigen activated, with 18 (67%) of the 27 infants requiring surgery for NEC and 10 (37%) dying from NEC or associated complications. Evidence of haemolysis (18 infants) and renal impairment (14 infants) were the common coexisting clinical features in the activated infants.

During the period of T or Tk antigen activation, 15 infants received low titre T FFP. Eleven (73%) had surgery and six (40%) died. Moderate to severe haemolysis (3+ spherocytes on blood film with hyperbilirubinaemia or hyperkalaemia) was seen in seven (47%). Eight infants had FFP given during early NEC but not while T or Tk antigen activated. Five (62%) had surgery and three died (37%). Four received only albumin, two underwent surgery, and one died. Moderate to severe haemolysis was seen in the infants who died. There was no clear trend in associating moderate to severe haemolysis, need for surgery, or mortality with the use of low titre T antibody plasma. Four infants with severe hyperbilirubinaemia had exchange transfusions with washed red cells resuspended in albumin. Three died either during or shortly after the exchange.

#### COMPARISON OF T AND Tk ANTIGEN ACTIVATED INFANTS

Of the T antigen or Tk antigen activated infants, five of 10 (50%) T antigen and 12 of 17

(71%) Tk antigen activated infants were born after the introduction of the routine testing of infants with suspected sepsis or NEC. Five T antigen activated infants died, three before the introduction of testing and two subsequently. Five Tk antigen activated infants also died, one before and four after the introduction of routine testing. The prevalence of haemolysis (7/10, 70% *vs* 11/17, 65% respectively), hyperbilirubinaemia (2/10, 20% *vs* 4/17, 24%), hyperkalaemia (3/10, 30% *vs* 4/17, 24%) and haematuria (3/10, 30% *vs* 7/17, 41%) were similar. There were no significant differences in incidence of renal impairment (maximum creatinine 93.3 (SD 24.6) *vs* 113.5 (43.4) mmol/l, respectively), the transfusion requirements of colloid (80 (34) *vs* 68 (42) ml/kg) and packed cells (43.5 (27) *vs* 49.5 (37.5) ml/kg) between the T and Tk antigen activated infants, respectively. Overall, more T antigen activated than Tk antigen activated infants died (5/10, 50% *vs* 5/17, 29%, respectively) or required surgery (8/10, 80% *vs* 10/17, 59%, respectively). The differences in outcome were not significant.

#### CASE-CONTROL STUDY OF ACTIVATED AND NON-ACTIVATED INFANTS

In the case-control study of infants with confirmed NEC diagnosed after the introduction of prospective testing of all infants with suspected sepsis or NEC, T and Tk antigen activated infants were matched with at least one weight and gestation matched control. With six infants no second match could be found. The details of 17 cases and 28 matched controls are summarised in tables 1 and 2. Cases and controls had similar mean gestations and birthweights. The T and Tk antigen activated infants did not differ significantly from the controls for any perinatal factor, including occurrence of abnormal umbilical arterial waveforms, respiratory distress syndrome, and patent ductus arteriosus (table 1). All 17 activated infants and 26 (93%) of the non-activated infants were fed before the development of NEC. While most infants received a mixture of expressed breast milk and formula feed, the same proportion (29%) of infants received breast milk only. Before the development of NEC, 14 of 17 (82%) infants with T or Tk antigen activation and 27 of 28 (96%) of matched controls received transfusions with either fresh frozen plasma or packed red cells.

Infants with T or Tk antigen activation had a significantly higher mortality than controls (table 2), although a similar number of surgical interventions was required. A trend to more hypotension treated with inotropes occurred in activated infants. Activated infants received significantly more colloid and bicarbonate. Blood film evidence of haemolysis was significantly more common in T and Tk antigen activated infants (71% *vs* 21%). There was no significant difference in the volume of transfused blood given during the course of NEC. Hyperkalaemia (serum  $K^+$   $\geq$  6.5 mmol/l) was more common in activated infants (47% *vs* 4%). Five activated infants and one control infant required an insulin and dextrose infusion for

Table 2 Outcomes of 17 T and Tk antigen activated infants compared with outcomes of 28 weight and gestation matched controls who developed NEC after September 1991

Variable	T/Tk antigen activated infants (%)	Matched control infants (%)	Odds ratios (95% confidence intervals)	p Value
Number of infants	17	28		
Died	6 (35%)	2 (7%)	7.1 (1.2, 40.8)	0.04*
Surgery for NEC	11 (65%)	14 (50%)	1.8 (0.5, 6.3)	0.37 NS
Haemolysis on blood film	12 (71%)	6 (21%)	8.8 (2.2, 35.0)	0.002*
Bilirubin $\geq 250$ $\mu\text{mol/l}$ with NEC	3 (18%)	1 (4%)	5.8 (0.5, 60.9)	0.14 NS
Exchange transfusion	1 (6%)	0 (0%)	5.2 (0.2, 134.8)	0.38 NS
Hyperkalaemia ( $\text{K}^+ \geq 6.5$ mmol/l)	8 (47%)	1 (4%)	24 (2.6, 219.2)	0.0008*
Haematuria ( $\text{UA} \geq 3+$ )	7 (41%)	1 (4%)	18.9 (2.1, 173.7)	0.003*
Anuria	6 (35%)	1 (4%)	14.7 (1.6, 137.1)	0.008*
Maximum creatinine (SD) ( $\mu\text{mol/l}$ )	104.6 (42.6)	74.4 (29.2)		0.02*
Hypotension treated with inotropes	12 (71%)	11 (39%)	3.7 (1.0, 13.5)	0.07 NS
Acidosis treated with bicarbonate	6 (35%)	2 (7%)	7.1 (1.2, 40.8)	0.04*
Colloid required (SD) (ml/kg)	69 (35)	31 (23)		0.0005*
Blood transfusions (SD) (ml/kg)	48 (33)	42 (28)		0.52 NS
Mean lowest platelet count ( $\times 10^9/\text{l}$ ) (SD)	152 (139)	228 (177)		0.14 NS
Platelet transfusion given	7 (41%)	5 (18%)	3.2 (0.8, 12.6)	0.16 NS
Leucopenia (white cells $< 5.0 \times 10^9/\text{l}$ )	9 (53%)	9 (32%)	2.4 (0.7, 8.2)	0.22 NS
Positive blood culture	6 (35%)	6 (21%)	2.0 (0.5, 7.7)	0.32 NS

CI, confidence interval; \* significant,  $p < 0.05$ .

hyperkalaemia. Significantly more haematuria, renal impairment with anuria, and higher maximum creatinine were seen in activated infants. Infants with T and Tk antigen activation had a non-significant trend towards worse haematological parameters, including more frequent and severe thrombocytopenia, and leucopenia. The subsequent incidences of retinopathy of prematurity, periventricular haemorrhage, and chronic lung disease were not different.

#### HISTORICAL STUDY OF THE EFFECT OF TESTING

Before the introduction of routine testing for T antigen activation in September 1991, 10 (9%) of the 110 infants with confirmed NEC were activated (five T and five Tk antigen). Seventeen (19%) of the 91 confirmed NEC infants were activated subsequently (five T and 12 Tk antigen). The incidence of surgical intervention in activated infants, seven (70%) before and 10 (65%) after, and mortality of four (40%) and six (35%), respectively, differed little between the two time periods.

Infants who developed confirmed NEC after September 1991 differed from infants born before with respect to several perinatal risk factors (table 3). Infants who developed confirmed NEC in the latter period had significantly lower mean gestations and non-significantly lower mean birthweights compared with infants from the earlier period (29.5 (3.8) weeks *vs* 28.3 (2.7),  $p = 0.01$ ; 1304 (644) g *vs* 1150 (459),  $p = 0.06$ ). They were

also more likely to be born to mothers with an antepartum haemorrhage (30 *vs* 13%), less likely to be born to mothers with hypertensive disease of pregnancy (11 *vs* 22%), more likely to have been born after antenatal steroids (74 *vs* 17%) and had less growth restriction (5% *vs* 15%). There were no significant differences in the prevalence of infants born to mothers with abnormal umbilical artery Doppler flows or of male infants. The prevalence of patent ductus and hyaline membrane disease were similar between the two time periods.

Table 3 documents the effect of the introduction of testing on mortality and incidence of surgery for NEC after September 1991. There was a significant increase in the overall mortality of all NEC infants (12/110 *vs* 22/91,  $p = 0.01$ ) and the number of NEC infants undergoing surgery (35/110 *vs* 51/91,  $p = 0.0006$ ). After adjustment for hypertensive disease of pregnancy, use of antenatal steroids, and fetal growth restriction, the trend to increase in mortality (adjusted odds ratios 1.8; 95% confidence intervals 0.9, 3.7) was no longer significant. After adjustment for hypertensive disease of pregnancy, antepartum haemorrhage, steroids and gestation the trend to increased incidence of surgery was also no longer significant (adjusted odds ratios 1.8, 95% CI 0.9, 3.7). Both regression models for mortality and surgery for NEC incorporated antenatal steroids as a confounder. If antenatal steroids were excluded from the analysis the

Table 3 Outcome of NEC infants before and after introduction of routine testing of all suspected NEC infants for T and Tk-antigen activation in September 1991

Outcome	Routine testing		Unadjusted odds ratio (95% CI)	p Value	Adjusted odds ratio (95% CI)	p Value
	Before	After				
No of NEC infants	110	91				
Birthweight (g) (mean (SD))	1304 (644)	1150 (459)		0.06		
Gestation (weeks) (mean (SD))	29.5 (3.8)	28.3 (2.7)		0.01		
Antepartum haemorrhage (n)	14 (13%)	27 (30%)		0.003		
Hypertensive disease of pregnancy (n)	24 (22%)	10 (11%)		0.04		
Growth restriction ( $< 10$ th percentile)	16 (15%)	5 (5%)		0.04		
Antenatal steroids (n)	19 (17%)	67 (74%)		$< 0.0001$		
Apgar score at 5 minutes (SD)	7.7 (1.5)	7.4 (1.7)		0.15		
Surgery for NEC	35 (32%)	51 (56%)	2.7 (1.5, 4.9)	0.0006	1.8 (0.9, 3.7)	0.1
Died	12 (11%)	22 (24%)	2.6 (1.2, 5.6)	0.01	2.0 (0.8, 5.2)	0.1

association between testing and higher mortality and surgery for NEC remained significant.

### Discussion

This is the largest reported series of T antigen activated infants. The ascertained incidence of T or Tk antigen activation in infants with confirmed NEC was 9% before the introduction of routine testing and 19% afterwards. All activated infants had confirmed NEC. The difference in incidence of T or Tk antigen activation is likely to have been due to the introduction of routine testing of infants with suspected NEC. A variable incidence of activation has been reported,<sup>21</sup> with Novak<sup>13</sup> screening 62 infants with NEC of whom 27% were T antigen activated, and Williams<sup>9</sup> screening all admissions over three years and finding that 11% of infants with NEC were T antigen activated. The incidence of surgical intervention and mortality among our activated infants was high and differed little between the two time periods in the activated infants identified. Our experience is similar to that of Seges,<sup>2</sup> who found 75% of infants with NEC and T antigen activation had perforated, and Klein,<sup>4</sup> who found 76% of T antigen activated infants required surgery. All strongly T antigen activated infants required surgery in Klein's study. In Williams' study<sup>9</sup> all eight infants who were T antigen activated required surgery.

There is little published evidence on the clinical course or outcome of Tk antigen activation in infants with NEC. Marshall<sup>18</sup> reported a single fatal case of Tk antigen activation in a 30 week gestation infant with NEC. We found no significant difference between infants with T and Tk antigen activation, although there was a trend to lower mortality and lower rates of surgery in the Tk antigen activated infants. Previous reports are predominantly of T antigen activated infants. Some of these might have been Tk antigen activated. We recommend testing with the full panel of lectins to differentiate.

The case-control study comparing activated with non-activated infants used cases from the period after the introduction of routine testing to avoid selection bias from the incomplete ascertainment of cases likely to have occurred before routine testing. Despite the use of low titre anti-T blood products while activated, T or Tk antigen activation was associated with higher mortality, need for surgery, evidence of haemolysis and renal impairment.

The management of infants with T or Tk antigen activation, many of whom are critically ill and require surgery, poses a major challenge to clinicians. Transfusion recommendations have included the use of plasma free products, washed red cells resuspended in albumin, and the use of low titre T antibody FFP and packed cells where necessary.<sup>1 2 9 13 19</sup> Haemolysis in our activated infants did not necessarily coincide with the use of FFP, although the incidence of haemolysis in these infants was high. Squire<sup>12</sup> reported four infants with NEC with severe haemolysis. Two were treated with exchange transfusion, including one that had

documented T antigen activation and survived. Williams<sup>9</sup> reported one infant in whom exchange transfusion with washed red cells resuspended in albumin, and subsequent transfusions resuspended in FFP, temporarily eliminated evidence of T antigen activation and reduced the plasma free haemoglobin concentration. In contrast, four of our infants with severe hyperbilirubinaemia had exchange transfusions with washed red cells resuspended in albumin and three died. The three infants who died also had severe hyperkalaemia: two received insulin and dextrose infusions. Hyperbilirubinaemia could be a combined result of endotoxin mediated and hypotension induced hepatic injury and haemolysis. Likewise, hyperkalaemia could be a result of haemolysis and renal impairment. Although the infants received exchange transfusions for hyperbilirubinemia and not for haemolysis, they illustrate the risks of exchange transfusion in critically ill infants.

Several authors have suggested routine testing for T antigen activation. Novak<sup>13</sup> found a significantly reduced incidence of post transfusion haemolysis (6% *vs* 24%) and death (5% *vs* 18%) from NEC in 62 infants who had been prospectively tested for T antigen activation compared with 66 historical controls who had not been tested. Novak's tested infants did not receive any plasma products if they were T antigen activated. Williams<sup>9</sup> found 10 infants with T antigen activation in a prospective study (which had a low 10% incidence of T antigen activation). Two activated infants received plasma products: one had massive haemolysis and died; the other infant had mild haemolysis. Avoidance of plasma products in all infants with suspected NEC or sepsis irrespective of T antigen status was suggested. Our approach was similar to Novak's in the use of low titre T antibody blood products and washed cells suspended in saline or albumin. In contrast to Novak, who found a significant historical decrease in mortality and surgery, we have not found any reduction in incidence of surgical intervention and mortality from NEC since the introduction of routine testing in September 1991. In fact, the opposite has occurred. Although antenatal steroids can reduce neonatal mortality and possibly NEC,<sup>22</sup> they were a significant risk factor for both mortality and surgery for NEC in our historical control study. Whether or not antenatal steroids were included in the analysis did not affect our results: that historical comparison found no evidence of benefit from testing. The increased use of antenatal steroids may have been associated with other perinatal risk factors that were not measured in this study. Furthermore, the improved morbidity and mortality seen in Novak's historical control study could have been due to factors other than the introduction of testing and avoidance of plasma containing products. None the less our hypothesis of reduced mortality and surgical morbidity by use of routine testing of at risk infants was not substantiated by our historical experience.

The alternative approach, as suggested by Williams,<sup>9</sup> is to avoid all plasma containing

products in infants with suspected sepsis or NEC. But this is more costly and time consuming and requires the use of albumin products and triple washed red cells in all infants at risk of NEC or sepsis where possible. This approach has the theoretical advantage of avoiding giving plasma containing anti-T which may persist and cause subsequent haemolysis in an infant who then becomes activated. However, in four of our infants who received albumin alone during the episode of NEC, two had surgery and one died. The infants who died had moderate to severe haemolysis. Most infants at risk of NEC, at some point before the onset of NEC, were given plasma products that were not screened for T antibody. Further studies are required to confirm the benefits of Williams' approach.

In conclusion, T and Tk antigen activation in infants with NEC is associated with an increased incidence of haemolysis, hyperbilirubinaemia, hyperkalaemia, renal impairment, surgery and death. T or Tk antigen activation may either reflect increased disease severity due to NEC, or be a major contributor to the severity of disease in NEC. Despite screening of all infants with suspected sepsis or NEC and the use of low titre T blood products in activated infants, two thirds of these T or Tk antigen activated infants will require surgery, with a significantly higher mortality than compared to those who are not activated. In a retrospective case controlled study, routine testing of at risk infants increased the detection rate of T and Tk antigen activation. A randomised controlled trial of testing in at risk infants, or of the use of low titre T plasma products in babies with NEC and T activation is warranted. The use of exchange transfusion as treatment of T or Tk antigen activation is also unconfirmed.

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## T and Tk antigen activation in necrotising enterocolitis: manifestations, severity of illness, and effectiveness of testing

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