Guidelines for the investigation and management of Transient Leukaemia of Down Syndrome

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Scope

Methodology

This guideline was compiled according to the British Society for Haematology (BSH) process at (http://www.bcshguidelines.com). The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org.

Literature review details. Ovid MEDLINE and Ovid EMBASE were searched systematically for publications in English from 1980 to the end of 2015 using the key words Transient Abnormal Myelopoiesis, Transient Myeloproliferative Disorder, Transient Leukaemia, and Down Syndrome. Specific searches relating to fetal disease and hepatic parameters were also performed. References from relevant publications were also searched.

Working group membership. The guideline group was selected to be representative of UK-based medical experts with invited representatives from the British Association of Perinatal Medicine and the Royal College of Paediatrics and Child Health.

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Review. Review of the manuscript was performed by the BSH Guidelines General Haematology Task Force, the BSH Guidelines Committee and the General Haematology sounding board of BSH. It was also placed on the members section of the BSH website for comment. Further comments were invited from a sounding board of the Childhood Leukaemia Clinicians' Network, the Childhood Cancer and Leukaemia Group (CCLG), the Royal College of Paediatrics and Child Health, the British Association of Perinatal Medicine (BAPM) and patient representatives identified through the Down Syndrome Association; these organisations do not necessarily approve or endorse the contents.

The objective of this guideline is to provide healthcare professionals with guidance on the investigation and management of patients with Transient Leukaemia of Down Syndrome (TL-DS). Individual patient circumstances may dictate an alternative approach. This is the first BSH guideline on this topic and is in date at time of publication. Any updates will be posted on the BSH Guidelines website (http://www.bcshguidelines.com).

Background

Between 5% and 30% of children with Down syndrome (DS) are born with transient leukaemia of Down syndrome (TL-DS), also known as transient abnormal myelopoiesis (TAM) and transient myeloproliferative disorder (TMD), a clonal disorder characterised by circulating megakaryoblasts and dysplastic changes in peripheral blood (PB) cells (Zipursky, 2003; Pine *et al*, 2007; Roberts *et al*, 2013). TL-DS is driven by mutations in the haematopoietic transcription factor gene *GATA1* and is only seen in conjunction with trisomy 21, either constitutional or acquired. TL-DS may present with overt clinical features but some cases are only identified through examination of the blood film and/or by *GATA1* mutation analysis (Klusmann *et al*, 2008; Roberts *et al*, 2013).

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Although many cases resolve without treatment, TL-DS results in early death in 15–23% cases and 20–23% of survivors will develop acute myeloid leukaemia of Down syndrome (ML-DS) in the first 4 years of life. Overall, TL-DS has an event-free survival of 63–68% (Massey *et al*, 2006; Klusmann *et al*, 2008; Gamis *et al*, 2011). Despite the very significant mortality and morbidity associated with the condition, care has not been standardised in the UK and many children do not receive the specialist care that is standard for all other paediatric malignancies. These guidelines aim to provide an evidence-based approach to the investigation and management of TL-DS and lay out clear treatment pathways to allow all children to receive the best possible care.

Definitions, clinical features and diagnosis of TL-DS

Terminology, nature and definition

TL-DS is a congenital leukaemia unique to neonates with DS or mosaic trisomy 21. The terms transient abnormal myelopoiesis (TAM) and transient myeloproliferative disorder (TMD) are also used to describe TL-DS but these terms can give a misleading impression of benignity. TL-DS displays many features of a malignant condition: TL-DS cells spread throughout the body, infiltrating the liver, pleural and pericardial spaces, skin and, to a lesser extent, the bone marrow. Despite its malignant nature, in the UK, care has not always been given by specialist Paediatric Oncology Principal Treatment Centres, potentially leading to mis- or delayed diagnosis, delayed treatment and avoidable death.

TL-DS is marked by the presence of an acquired N-terminal mutation in exon 2 or exon 3 of the key haematopoietic transcription factor gene *GATA1*, resulting in a truncated GATA1 protein (GATA1s) (Groet *et al*, 2003; Hitzler *et al*, 2003; Mundschau *et al*, 2003; Rainis *et al*, 2003; Xu *et al*, 2003; Ahmed *et al*, 2004; Alford *et al*, 2011). Paired TL-DS and ML-DS samples show the same *GATA1* mutation(s), indicating that they are clonally linked conditions (Ahmed *et al*, 2004; Yoshida *et al*, 2013). *GATA1* mutations are not detected in remission samples after treatment of ML-DS nor are they present in other DS and non-DS leukaemias (Wechsler *et al*, 2002). Furthermore, *GATA1* mutation(s) are not leukaemogenic in cells that are not trisomic for chromosome 21 (Hollanda *et al*, 2006). Studies using next generation sequencing (NGS) indicate that cases classified clinically as TL-DS (Yoshida *et al*, 2013) or by blast % (>10%; Roberts *et al*, 2013) all have detectable *GATA1* mutations. The failure to demonstrate *GATA1* mutations in clinically suspected TL-DS is likely to be due to one or more technical factors (e.g. a large *GATA1* deletion, lack of assay sensitivity or a sample with a low blast %), although some cases are reported where a mutation cannot be demonstrated even after extensive investigation (Schifferli *et al*, 2015).

The World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues (Swerdlow *et al*, 2008) recognises the unique clinical and molecular features and the central role of GATA1, and defines TAM (TL-DS) as 'increased peripheral blood blast cells in a neonate with Down syndrome'. No definition of increased peripheral blasts is offered. The Oxford-Imperial Down Syndrome Cohort Study (OIDSCS), which included a systematic examination of blood findings in neonates with DS together with sensitive *GATA1* mutational analysis, found that 98% of neonates with DS had circulating blasts, the great majority of whom had no clinical features of TL-DS and no detectable *GATA1* mutation (Roberts *et al*, 2013). Of note, no neonate without a detectable *GATA1* mutation had either clinical features of TL-DS or subsequently developed ML-DS (Roberts *et al*, 2013).

For these reasons, and as further discussed below, we recommend that, in keeping with other myeloid leukaemias in the WHO Classification, TL-DS is primarily defined on a genetic basis – the presence of a *GATA1* mutation in a neonate with DS or mosaic DS – combined with an increased blast count (see below) or features suggestive of TL-DS.

Blast count threshold. There is no internationally agreed definition of a percentage blast threshold that constitutes 'increased peripheral blood blast cells'. The only prospective study of neonates with DS to evaluate the clinical significance of the blast percentage in neonates with DS is the OIDSCS (Roberts *et al*, 2013). The interim analysis of the first 200 neonates enrolled in the study, supported by the recently updated analysis, has shown that a threshold of >10% peripheral blood blasts in the first week of life identifies all neonates with clinical features of TL-DS (Roberts *et al*, 2013; Bhatnagar *et al*, 2016). However, some neonates with DS with blasts >10% do not have a *GATA1* mutation even when very sensitive (NGS)-based methods are used. Using the updated Oxford study data, the sensitivity and specificity of blasts >10% (for the presence of *GATA1* mutations) is 74% and 81% respectively (Bhatnagar *et al*, 2016; and unpublished data). Higher blast thresholds are likely to be more specific for TL-DS based on previously published retrospective studies and on the OIDSC study. In the OIDSC study all neonates with blasts >20% had a *GATA1* mutation. Therefore, current data suggest that setting a blast threshold of >10% will identify more cases of TL-DS and that *GATA1* mutation analysis is particularly important in neonates with blasts of 10–20% to prevent over-diagnosis of TL-DS. Blast count assessment requires careful examination of a peripheral blood film in the first week of life, ideally in the first 3 days of life, by a haematologist experienced in reviewing neonatal blood films. We recommend referral of blood films from any cases with suspicion of TL-DS for morphology review by a paediatric haematologist. Automated blast counts are not accurate and blasts are often missed. Blast count assessment after the first week of life may underestimate the prevalence of disease as, in our experience, the blast % often falls rapidly after birth and we recommend blast count assessment as soon as possible after birth to prevent delay in diagnosis of clinically relevant and life-threatening cases of TL-DS. Care should also be taken in neonates with intrauterine growth restriction (IUGR) or other history of placental insufficiency (e.g. maternal hypertension, pre-eclampsia or diabetes mellitus) as these babies may have lower blast counts despite large mutant *GATA1* clones.

Clinical features of TL-DS

From their origin in the fetal liver, megakaryoblastic TL-DS cells can spread locally, spill into the peripheral blood and infiltrate throughout the liver as well as distant tissues. This usually manifests as enlargement of the liver; as malignant effusions in pleural and pericardial spaces; and/or as a papular or vesicopustular rash due to deposits containing TL-DS blast cells in the skin. Skin nodules in TL-DS also occur but reports are rare (Winckworth *et al*, 2012). Splenomegaly is found in 30% of cases, although this is often due to portal venous obstruction (Gamis & Smith, 2012) as splenic infiltration is rarely reported (Yagahashi *et al*,1995; Smrcek *et al*, 2001). Thus, TL-DS can present with a spectrum of abnormalities ranging from a few circulating blast cells in an otherwise well neonate to hyperleucocytosis, hepatic fibrosis and multi-organ failure (Massey *et al*, 2006; Klusmann *et al*, 2008; Muramatsu *et al*, 2008; Gamis *et al*, 2011).

No single clinical feature is entirely specific to TL-DS because each of these features may also occur in the absence of TL-DS (see Table I). However, there are several characteristic features that are seen relatively frequently in TL-DS but are uncommon in DS neonates without *GATA1* mutations, including organomegaly, hepatopathy (raised transaminases with conjugated hyperbilirubinaemia), skin rash, pericardial and pleural effusions, extreme leucocytosis and coagulopathy (Klusmann *et al*, 2008; Roberts *et al*, 2013). Presence of one or more of these features in the absence of a clear alternative explanation should lead to the early consideration of a diagnosis of TL-DS.

Morphology, immunophenotyping and bone marrow examination

TL-DS originates from abnormal megakaryocyte-erythroid precursors in the fetal liver (Chou *et al*, 2008; Tunstall-Pedoe *et al*, 2008; Roy *et al*, 2012). Circulating blast cells are pleomorphic, often having prominent nucleoli and basophilic, blebbed cytoplasm, in keeping with their erythroid-megakaryocytic origin, and megakaryocyte fragments are often a prominent feature (Fig 1) (Roberts *et al*, 2013). Immunophenotypically they have a phenotype distinct from other leukaemias, showing variable co-expression of stem cell markers (CD34 and CD117), myeloid markers CD33/CD13 and platelet glycoproteins (CD36, CD42 and CD61), as well as aberrant expression of CD56 and CD7 and low expression of CD11a (Langebrake *et al*, 2005; Klusmann *et al*, 2008; Boztug *et al*, 2013). The immunophenotype of blast cells in neonates with DS (CD45^{weak}CD34+/-CD33+/-CD36+CD7+/-) is distinct from that of blast cells in neonates without DS where these antigens are not co-expressed. However, no way of distinguishing *GATA1*-mutated blast cells from blasts without *GATA1* mutations has yet been reported in DS (Roberts *et al*, 2013). Bone marrow examination is not generally useful in TL-DS: blast cells are believed to originate in the liver and marrow blasts are variable and less prevalent than in peripheral blood. Bone marrow involvement does not correlate with disease severity (Massey *et al*, 2006; Klusmann *et al*, 2008; Gamis *et al*, 2011).

Recommendations

- **Transient leukaemia of Down syndrome (TL-DS) should be defined as the presence of a *GATA1* mutation together with a peripheral blood blast percentage >10% and/or**

Table I. Clinical and haematological features of TL-DS (Based on data from Roberts *et al*, 2013; Klusmann *et al*, 2008; unpublished data).

Clinical feature	TL-DS (% of cases*)	Silent TL-DS (% of cases*)	Neonate with DS and no *GATA1* mutation (% of cases*)
Hepatomegaly	40	<5	4
Splenomegaly	30	<1	<1
Rash	11	<1	1
Pericardial/pleural effusion	9	<1	<1
Jaundice plus one or more of the above	70	63	54
Jaundice alone	~20	20	~50
None of the above	~10	40	~40
Abnormal LFTs	25	<10	<10
Abnormal coagulation	10–25	~5	~5
Anaemia (Hb < 130 g/l)	5–10	<5	1–5
Thrombocytopenia (platelet count <150 × 10^9/l)	50	50	50
Thrombocytosis (platelet count >600 × 10^9/l)	1–2	<1	<1
Leukocytosis (leukocyte count >26 × 10^9/l)	~50	10	10–15
Neutrophilia (neutrophil count > 14·4 × 10^9/l)	10–15	5	20
Circulating blast cells >10%	100	0	2

DS, Down syndrome; Hb, haemoglobin; LFTs, liver function tests; TL-DS, transient leukaemia of Down syndrome.

*In each case '% of cases' refers to the percentage of patients in each of the 3 groups (TL-DS, Silent TL-DS, Neonate with DS but found to have no *GATA1* mutation) which has the clinical feature listed in the first column. Note that ~98% of all DS neonates have some circulating blast cells even if they do not have a *GATA1* mutation.

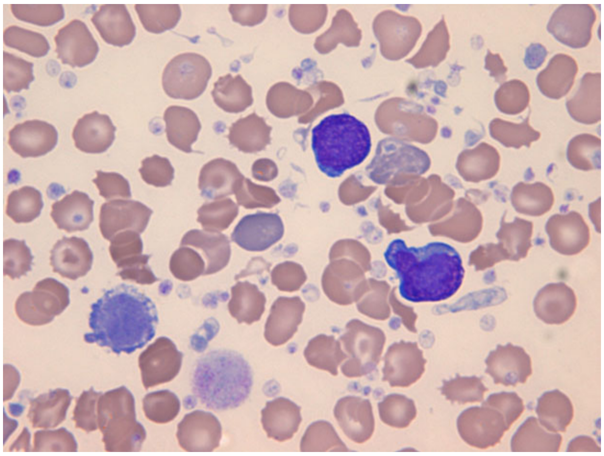

Fig 1. Typical appearances of TL-DS in peripheral blood of a neonate with Down syndrome. Photomicrograph from a blood film of a neonate with transient leukaemia of Down syndrome showing blast cells, platelet anisocytosis and a megakayocyte fragment (arrow).

clinical features suggestive of TL-DS in a child with Down syndrome (DS) or mosaic trisomy 21 (Grade 1B).

- **All neonates with known, or a high suspicion of, DS should be examined for features suggestive of TL-DS (organomegaly, cholestasis and hepatopathy, skin rash, pericardial and pleural effusions). In addition, they should have a full blood count and blood film requested in the first 3 days of life and a formal assessment of the peripheral blood blast cell percentage performed by a haematologist with experience in reviewing neonatal blood films. Babies in whom clinical examination and blast cell percentage indicate that TL-DS is likely should have additional tests considered: liver function tests including conjugated bilirubin if the baby has significant jaundice, chest X-ray, echocardiogram and abdominal ultrasound (Grade 1B).**
- **Any neonate with a blast percentage >10% and/or clinical features suggestive of TL-DS should be discussed urgently with the regional Paediatric Oncology Principal Treatment Centre and a peripheral blood sample sent for *GATA1* mutation analysis (Grade 1A).**
- **Any child who did not have a peripheral blood blast cell percentage performed in the first 3 days of life or in whom there was significant intra-uterine growth retardation (when blast counts may be suppressed) should be considered to be still at risk of clinical problems of TL-DS in the first 4-8 weeks of life and should be monitored accordingly. *GATA1* mutation analysis should be considered (Grade 1B).**

Silent TL-DS and screening for *GATA1* mutations

Currently available methodologies for *GATA1* mutation screening are direct Sanger sequencing, denaturing high performance liquid chromatography (dHPLC) (WAVE®) (Ahmed *et al*, 2004; Alford *et al*, 2011) and NGS (Roberts *et al*, 2013). Sensitivities for the different techniques are: direct Sanger sequencing 10–30%, dHPLC 2–10% and various methods of NGS 0·3–2%. All of these methods have technical limitations, advantages and disadvantages but direct Sanger sequencing and dHPLC are not reliably sensitive enough to detect small mutant *GATA1* clones (<10%) that may be clinically significant (i.e. that may lead to subsequent ML-DS) (Roberts *et al*, 2013). Using NGS, the high prevalence of mutant *GATA1* clones in neonates with DS (18/88 DS neonates) reported from the OIDSCS (Roberts *et al*, 2013) has now been confirmed on a larger sample size from the same study (82/267, 30·7%, Bhatnagar *et al*, 2016).

The recent OIDSCS found that at least half of DS neonates with *GATA1* mutations do not have blast percentages >10% and have no clinical features of TL-DS. The prevalence of *GATA1* mutations in DS neonates with blast percentages of 1–10% is around 20% though none of these children developed any clinically significant complications from TL-DS. The combination of one or more truncating *GATA1* mutation with no increased blast cell percentage in a neonate with DS has been termed Silent TL-DS or Silent TAM (Roberts *et al*, 2013; Bhatnagar *et al*, 2016).

Transformation to ML-DS

One neonate with Silent TL-DS has so far been reported to have transformed to ML-DS (Roberts *et al*, 2013) but unpublished data from the Oxford study show a rate of transformation of <3%, much lower than the rate seen in clinical TL-DS (10-30%) (Massey *et al*, 2006; Klusmann *et al*, 2008; Gamis *et al*, 2011; Flasinski *et al*, 2017; OIDSCS, unpublished data). Interim analysis of the prospective OIDSCS has found no cases of ML-DS in neonates with DS without a *GATA1* mutation detectable by NGS at birth (Bhatnagar *et al*, 2016). Together these data indicate that screening every child with DS at birth with a sensitive method for detecting *GATA1* mutations should identify all children at risk of subsequent ML-DS. However, there is currently no evidence of the clinical benefit and cost effectiveness of such an approach.

Recommendations

- **The term silent TL-DS should be used where there is a *GATA1* mutation and a peripheral blood blast percentage ≤10% in the first week of life in a neonate with DS or mosaic trisomy 21. These babies do not appear to be at risk from TL-DS and are at low risk of transformation to acute myeloid leukaemia. Screening for *GATA1* mutations is therefore not routinely recommended when the peripheral blood blast percentage is ≤10%, except in those cases where the neonatal blast percentage was not assessed or is deemed unreliable (Grade 2B).**

Table II. Rates of early death and ML-DS in TL-DS patients.

	Massey *et al* (2006) $n = 47$	Klusmann *et al* (2008) $n = 146$	Muramatsu *et al* (2008) $n = 70$	Gamis *et al* (2011) $n = 135$	Total $n = 398$
All early deaths	8 (17%)	22 (15%)	16 (23%)	29 (21%)	75 (19%)
TL-DS related deaths*	8 (17%)	13 (9%)	15 (21%)	14 (10%)	39 (10%)
Non-TL-DS related deaths	0	9 (6%)	1 (1·4%)	15 (11%)	25 (6%)
ML-DS†	9 (23%)	29 (23%)	12 (22%)	21 (20%)	71 (22%)

ML-DS, acute myeloid leukaemia of Down syndrome; TL-DS, transient leukaemia of Down syndrome.
*The commonest reported cause of death related to TL-DS was severe liver dysfunction with or without liver failure and/or liver fibrosis.
†Number and (%) of surviving children with TL-DS who later developed ML-DS.

- ***GATA1* mutation analysis should be performed in an accredited laboratory using a properly standardised, high sensitivity assay (Grade 1A).**

Outcomes, risk factors for early death, and treatment of TL-DS

Outcomes

Although most cases of TL-DS resolve spontaneously without sequelae, prospective large-scale studies of clinical TL-DS report an early mortality of 15–23% (Massey *et al*, 2006; Klusmann *et al*, 2008; Muramatsu *et al*, 2008; Gamis *et al*, 2011). Taken together, in the three prospective studies of TL-DS combined with the large Japanese retrospective study, 75/398 neonates diagnosed with TL-DS (19%) died within 6 months (See Table II). This compares with 8% mortality in the first year after diagnosis of all cancer in childhood in the UK in 2009 (Stevens, 2013). By type of cancer, one-year mortality rates in the UK varied from <1% for retinoblastoma to 19% for acute myeloid leukaemia (AML) (Stevens, 2013). This suggests that TL-DS has an early mortality in excess of any other childhood cancer in the UK.

The predominant cause of TL-DS-related death is a progressive hepatopathy with cholestasis, leading to fulminant hepatic fibrosis, disseminated intravascular coagulation (DIC) and multiorgan failure. Postmortem findings show diffuse intralobular hepatic fibrosis with extensive extramedullary haematopoiesis and a prominent infiltrate of megakaryoblasts (Miyauchi *et al*, 1992). Death due to hepatic fibrosis is reported in 5–17% of cases, 10% overall (Massey *et al*, 2006; Klusmann *et al*, 2008; Muramatsu *et al*, 2008; Gamis *et al*, 2011).

Non-hepatic deaths directly attributable to TL-DS occur in 3% of affected children, most often due to cardiorespiratory failure associated with malignant pericardial and pleural effusions, hydrops fetalis, renal failure and infection (Massey *et al*, 2006; Klusmann *et al*, 2008; Muramatsu *et al*, 2008; Gamis *et al*, 2011). Early death in neonates with TL-DS may also occur due to causes not directly attributable to TL-DS, e.g. secondary to severe cardiac disease or other congenital abnormalities (Klusmann *et al*, 2008; Gamis *et al*, 2011).

Risk factors for early death

Factors predictive of early death are summarised in Table III. The factor most consistently associated with early death in the 3 large studies of TL-DS was hyperleucocytosis (Massey *et al*, 2006; Klusmann *et al*, 2008; Gamis *et al*, 2011). In 2 of the studies a white cell count $>100 \times 10^9$/l was significantly associated with early death (Klusmann *et al*, 2008; Gamis *et al*, 2011); in the third study (Massey *et al*, 2006) the mean white cell count of the infants with TL-DS who died early was $100{\cdot}2 \times 10^9$/l compared to $39{\cdot}8 \times 10^9$/l in the survivors.

A second consistent factor associated with early death in TL-DS is severe liver disease. In the study by Massey *et al* (2006), transaminase levels were significantly higher in infants with TL-DS who died early compared to survivors and all of the infants with TL-DS who died early had evidence of liver failure and DIC. Similarly, in the Klusmann *et al* (2008) study, all 7 patients with TL-DS with hepatic fibrosis died. This study also found a significantly higher frequency of hydrops fetalis, ascites and coagulopathy in the children with TL-DS who died early compared to those who did not.

The Children's Oncology Group (COG) (Gamis *et al*, 2011) used a combination of the presence or absence of what they defined as life-threatening symptoms (LTS, see Table IV

Table III. Risk factors for early death on multivariate analysis.

	Hazard ratio	*P* value
Klusmann *et al* (2008)		
Preterm delivery (<37/40)	4·1	0·032
Ascites	4·6	0·006
White blood cell count $>100 \times 10^9$/l	5·0	0·003
Bleeding diathesis	11·0	<0·001
Cytarabine treatment	0·11	<0·001
Muramatsu *et al* (2008)		
Preterm delivery (<37/40)	3·6	0·03
White blood cell count $>100 \times 10^9$/l	3·0	0·02
Direct bilirubin ≥83 μmol/l	5·4	0·05
Gamis *et al* (2011)		
Hepatomegaly	2·8	0·048
White blood cell count $>100 \times 10^9$/l	2·2	0·101
Black race	3·5	0·013

Table IV. Life Threatening Symptoms – Indications for Treatment of TL-DS (Adapted from Gamis *et al*, 2011).

Multi-organ failure
White blood cell count >100 × 10^9/l or leucostasis
Hepatopathy (conjugated bilirubin >83 μmol/l, ascites or massive hepatomegaly)
Hepatosplenomegaly (beyond umbilicus or causing respiratory or feeding compromise)
Hydrops fetalis
Pleural or pericardial effusions
Renal failure
Disseminated intravascular coagulation/coagulopathy with bleeding

and later discussion) and the presence of absence of hepatomegaly to retrospectively divide patients into three groups: high risk (any LTS), moderate risk (hepatomegaly with no LTS) and low risk (no LTS or hepatomegaly). One-year overall survival for the three groups was 45%, 77% and 92% respectively, but most significantly, almost all deaths in the low and moderate risk groups were not deemed to be related to TL-DS. Thus, these data suggest that the presence of LTS should prospectively identify almost all children at risk of TL-DS related early death.

Treatment of TL-DS

Cytarabine. TL-DS and ML-DS blast cells are extremely sensitive to cytarabine (Taub *et al*, 1999; Zwaan *et al*, 2002). This means that very low doses of cytarabine can be successfully used to drive blast clearance in TL-DS. Al-Kasim *et al* (2002) reported that of 9 patients identified to have life-threatening hepatic disease, 3 patients were treated with low dose cytarabine (0·5–1·5 mg/kg twice daily for 5–7 days) and all survived. One further patient was commenced on cytarabine *in extremis* but died within 24 h; all 5 who did not receive treatment died (Al-Kasim *et al*, 2002).

The Berlin-Frankfürt-Münster (BFM) group recommended treatment with cytarabine (0·5–1·5 mg/kg for 3–12 days) for any patients with TL-DS presenting with clinical impairment due to thrombocytopenia, signs of cholestasis or liver dysfunction, or high white cell count (>50 × 10^9/l). Out of 146 children, 28 received treatment with cytarabine. Even though the treatment group included large numbers of children requiring intensive care (46% vs 20% in non-treatment group) and several with hepatic fibrosis (10% vs. 3%), survival in the 2 groups was very similar (5-year overall survival 78 ± 8% vs. 85 ± 3%, $P = 0{\cdot}44$), suggesting that treatment was beneficial. Consistent with this, when analysis was confined to those patients identified as high risk using multivariate analysis (high white cell count, ascites, preterm delivery, bleeding or failure to spontaneously remit), the cumulative incidence of death was 24% in the treatment group vs. 72% for the non-treatment group ($P < 0{\cdot}001$) (Klusmann *et al*, 2008). Further evidence for the efficacy of low dose cytarabine comes from a recent presentation of the TAM-10 study from Japan: babies with a white cell count >100 × 10^9/l showed a clear improvement in one-year survival when treated with cytarabine – 78·3% vs. 38·5% for those not treated ($P = 0{\cdot}009$) (Muramatsu *et al*, 2015).

The COG identified 38 of 135 patients as having LTS (see Table IV) and 24 patients were given cytarabine as a continuous infusion at a dose of 3·33 mg/kg/day for 7 days (Gamis *et al*, 2011). Ninety-six percent of patients suffered grade 3 or 4 toxicities, perhaps reflecting the use of higher dose than in other studies. Fifty-one percent of the treatment group survived.

It is the authors' experience that there is often reluctance to commence chemotherapy for a condition that may spontaneously resolve and that this delays the timely institution of potentially life-saving treatment despite evidence of low toxicity with daily cytarabine doses of 0·5–1·5 mg/kg.

Exchange transfusion and leukapheresis. Exchange transfusion is a relatively common intervention in Neonatal Intensive Care Units. In the context of TL-DS, it may have value in rapidly reducing white cell counts in patients with leucostasis though it would not be predicted to treat other complications. In the COG study, 10 of the LTS group were initially treated with exchange transfusion or leukapheresis: 2 needed no further treatment, 2 died before receiving further treatment, 1 had repeated exchange and 5 later received cytarabine (Gamis *et al*, 2011). Hayasaka *et al* (2015) recently reported their experience of exchange transfusion in TL-DS patients: 5 babies received exchange transfusion at a median of 2 days of age, dropping the white cell count from a mean of 143 × 10^9/l to a mean of 21 × 10^9/l with clear short term clinical improvement in all cases, although 2 subsequently died and 2 more progressed to AML in the first year.

Low dose cytarabine to prevent ML-DS

The BFM group recently presented their data from the AML-BFM TMD Prevention 2007 trial, a prospective study looking at the use of low dose cytarabine to prevent ML-DS. Neonates with a clinical syndrome of TL-DS and any babies with persistent disease detectable by flow or molecular minimal residual disease were treated with a course of cytarabine at 1·5 mg/kg/day for one week (Flasinski *et al*, 2017). While there was a non-significant trend towards improved survival (80+/−6% vs. 67+/−7%, $P = 0{\cdot}1$) in those with clinical TL-DS compared to a historical control, there was no apparent reduction in the cumulative incidence of ML-DS (19+/−6% vs 22+/−4%, $P = 0{\cdot}95$). This result is in keeping with the COG experience, which again found no protective role from low dose cytarabine with regards to incidence of ML-DS (Gamis *et al*, 2011).

Indications for treatment of TL-DS

Given the high mortality figures in TL-DS, the evidence of benefit of treatment in some groups, the knowledge that most cases will spontaneously resolve, and the lack of evidence that treatment prevents subsequent development of ML-DS, it is clear that, based on our current knowledge, some, but not all patients should be treated with low dose cytarabine. Thus, the main therapeutic question in TL-DS is who, and when, to treat.

The BFM Group's indications to treat were "clinical impairment due to thrombocytopenia, signs of cholestasis or liver dysfunction, or high white cell count (>50 × 10^9/l)" (Klusmann *et al*, 2008). Of these, we now know that thrombocytopenia is not only not associated with early death, but is actually no more common in TL-DS than it is in neonates with DS and no *GATA1* mutation (Roberts *et al*, 2013); we would therefore not consider thrombocytopenia as an indication to treat. Cholestasis and liver dysfunction are predictive of hepatic fibrosis and death (Massey *et al*, 2006; Hirabayashi *et al*, 2007; Klusmann *et al*, 2008) and are therefore reasonable criteria to use, although the specific cut-offs used by the BFM – conjugated bilirubin >256 μmol/l (36 times the upper limit normal) and transaminases (1·5 times the upper limit normal) – appear high and low respectively – see below.

The COG's indications to treat were the presence of LTS (see Table IV): hyperviscosity, hepatopathy, renal failure, hydrops fetalis, coagulopathy with bleeding, respiratory compromise due to organomegaly and cardiac failure not due to congenital anomalies, all these being signs of advanced, potentially fatal, TL-DS; a white cell count >100 × 10^9/l has been repeatedly associated with early death. Taken together, the presence of LTS was associated with a one-year survival of 45% and predicted all but one of the TL-DS related deaths. Thus, these indications appear to be justified.

When to treat hepatic disease. Evidence of hepatic disease is often one of the key determinants of when to treat but tight definitions of hepatopathy/hepatic dysfunction are lacking. The typical pattern of progressive hepatic disease is that of hepatomegaly with a rising conjugated bilirubin, often accompanied by increasing transaminase levels, later leading to ascites and DIC. However, the pattern of hepatic disease is variable and may already present at birth together with ascites and coagulopathy. There is often worsening hepatic function in the context of an improving blood count (Park *et al*, 2014). However, hepatomegaly alone is relatively common – Gamis *et al* (2011)reported that hepatomegaly was present in 50% of TL-DS patients who were not felt to warrant treatment and moderate hepatomegaly in the absence of LTS defined an intermediate risk group who were at low risk of early death directly attributable to TL-DS. Massive hepatomegaly (beyond the umbilicus) was almost completely confined to the high-risk group.

Miyauchi *et al* (1992) reported a series of 8 cases of TL-DS, of whom 6 died due to hepatic disease. In those who died, mean conjugated bilirubin at presentation was 84 μmol/l (42–122), progressing to a mean of 319 μmol/l (158–400) at the time of death.

Muramatsu *et al* (2008) reported a large retrospective series and found that the presence of a conjugated bilirubin >83 μmol/l was strongly associated with early death on both univariate and multivariate analysis – hazard ratios 6·1 (P = 0·002) and 5·5 (P = 0·005) respectively.

Park *et al* (2014) reported their centre's experience of 25 infants with TL-DS over a 12-year period with particular reference to the natural history of liver disease. Of note, all infants with TL-DS, barring the child who died on day one, developed raised conjugated bilirubin levels, peaking on a median of day 17 at a time when peripheral blood blast counts were falling. Five had a peak conjugated bilirubin >83 μmol/l: three suffered early death, one improved after cytarabine and the other had a diagnosis of 'non-syndromic paucity of interlobular bile ducts'. Interestingly, a rise in transaminases was neither sensitive nor specific for TL-DS hepatic disease.

Hirabayashi *et al* (2007) used their own institution's experience of hepatic disease in TL-DS to assess potential scoring systems. Of 25 patients diagnosed, three (12%) died. They proposed treating any babies who had two out of the following criteria: a modified Child-Pugh Score >5·5 (score 1–3 on conjugated bilirubin, ascites and coagulopathy); hyaluronic acid >500 u/ml; hepatomegaly resulting in mechanical ventilation or tube feeding; fibrosis on liver biopsy. However, it is not clear how much this adds given that ascites, a high conjugated bilirubin, coagulopathy and hepatomegaly causing respiratory failure, could all be considered criteria to treat in isolation. Hyaluronic acid assessment is not universally available in the UK, and biopsy-defined fibrosis has previously been associated with death in 100% of babies.

Monitoring response to treatment and repeated courses of cytarabine. Treated children should be closely monitored both for evidence of ongoing disease and because of the risks of cytarabine-associated neutropenia and sepsis. In some cases, a single course of cytarabine is not sufficient to control TL-DS entirely. Peripheral blast counts respond quickly to therapy but liver disease typically progresses in the first weeks of life (Park *et al*, 2014) and can be refractory to an initial course of cytarabine. In particular, liver disease often takes a different natural history to the peripheral blast count. Repeat courses of cytarabine should be carefully considered to achieve control where severe liver dysfunction persists and it should be noted that hepatomegaly will often take months to resolve and is not necessarily indicative of active disease. Study data are limited on repeated courses – in the COG paper only 1/24 received a second course though 13/24 died from disease progression or sepsis (Gamis *et al*, 2011).

Recommendations

- **All neonates with TL-DS or presumed TL-DS should be urgently assessed and then watched closely for the development of life threatening symptoms (LTS) (See**

Table IV) and should have regular laboratory monitoring of full blood count (FBC), blood film and liver function tests until these normalise. Any child with LTS should be urgently considered for treatment with cytarabine. Specific parameters of hepatic dysfunction that should prompt initiation of treatment include a conjugated bilirubin of >83 μmol/l, ascites and massive hepatomegaly (beyond the umbilicus and/or compromising respiratory function or feeding) (Grade 1B).

- **When treatment is indicated, cytarabine should be given without delay at a dose of 1–1·5 mg/kg/day for 5–7 days either intravenously or subcutaneously (Grade 1B). Treated children should be closely monitored because of the risks of cytarabine-associated neutropenia and sepsis. Liver disease takes a different natural history to the peripheral blast count and repeated courses of cytarabine can be considered to achieve control where severe liver dysfunction persists. Exchange transfusion and leukapheresis may be of use in acute count reduction but should not be considered definitive treatment (Grade 2C).**
- **There is no evidence to support the routine use of cytarabine in neonates solely to prevent later development of acute myeloid leukaemia of Down syndrome (ML-DS) and this is not recommended (Grade 2A).**

Monitoring for resolution of TL-DS and development of Ml-DS

Monitoring for resolution of TL-DS

In those cases of TL-DS where there are no LTS, it is reasonable to monitor without treatment as almost all cases will spontaneously resolve: the COG found 106 of 108 such children showed spontaneous disappearance of the peripheral blast cells at a median of 36 days (range 2–126), the other 2 infants developed LTS and required treatment (Gamis *et al*, 2011). However, some children develop ML-DS without ever showing normalization of counts (Klusmann *et al*, 2008); no study has shown any spontaneous resolution after 6 months of age (Massey *et al*, 2006; Klusmann *et al*, 2008; Gamis *et al*, 2011).

Studies of clinical TL-DS have shown a risk of progression to ML-DS of 20–23% amongst survivors: overall 71 of 323 children (22%) who survived TL-DS developed ML-DS (Massey *et al*, 2006; Klusmann *et al*, 2008; Muramatsu *et al*, 2008; Gamis *et al*, 2011). The OIDSCS, which used more sensitive techniques for *GATA1* mutation detection, estimated that the risk of transformation in children with DS who had a *GATA1* mutation detected at birth (~30% of all neonates with DS) was closer to 5–10% (Roberts *et al*, 2013). There is no evidence that treatment with cytarabine prevents progression to ML-DS (Gamis *et al*, 2011).

Minimal residual disease monitoring for relapse/ML-DS

Following resolution of clinical signs of TL-DS, the full blood count (FBC) and blood film will usually return to normal. Thereafter, a small proportion of children will have persistent FBC abnormalities or will relapse within the first 3 months of life with variable pancytopenia. In our experience, *GATA1* mutation analysis can be useful to establish the diagnosis in these children. However, the value of monitoring all cases of TL-DS for the persistence of the *GATA1* mutation and/or of quantitative assessment of the size of any residual *GATA1*-mutant clone in TL-DS or silent TL-DS has not yet been demonstrated. Small studies have evaluated flow cytometric monitoring of persistent blast cells based on identifying a distinct leukaemia-associated immunophenotype (Klusmann *et al*, 2008) or using mutation-specific quantitative real time polymerase chain reaction (Hitzler & Zipursky, 2005; Pine *et al*, 2005). There is no evidence at present to show that either of these approaches is predictive of relapse. In addition, up to 25% of cases of TL-DS have been shown to have multiple mutant *GATA1* clones (up to six) at presentation (Ahmed *et al*, 2004; Alford *et al*, 2011; Roberts *et al*, 2013; Yoshida *et al*, 2013) and ML-DS may develop from the smaller ('minor') rather than the larger ('major') *GATA1*- mutant clone (Yoshida *et al*, 2013).

Monitoring for ML-DS

Given that ML-DS has a peak incidence in the second year of life and is rare after the age of 4 years (Hasle *et al*, 2000; Zipursky, 2003; Uffmann *et al*, 2017), monitoring of children with TL-DS can be safely discontinued by the age of 4 years if the FBC is normal. ML-DS often has an indolent presentation with a myelodysplastic syndrome-like picture with progressive pancytopenia, usually with a low percentage of circulating blasts, for many months before increasing blast cells mark the development of AML. Often the first, and only, sign of incipient ML-DS in an infant with a history of TL-DS is a falling platelet count. All such cases will progress if left untreated and the recommendation is to treat all cases as ML-DS (Zipursky, 2003; Swerdlow *et al*, 2008). There are no published studies to establish the optimum frequency of monitoring for ML-DS. However, given that most cases of ML-DS will present before age 2 years (Hasle *et al*, 2000; Uffmann *et al*, 2017), there is a case for more frequent monitoring up to this age. Furthermore, the indolent nature of evolution to ML-DS in most cases means that a FBC every 3 months is likely to identify incipient ML-DS promptly. Similarly, as most cases of ML-DS will have already presented by age 2 years, we suggest that it is reasonable to reduce the frequency of FBC monitoring after this age if the FBC is normal. Any significant blood count abnormality, particularly thrombocytopenia, should prompt *GATA1* mutation analysis and early consideration of a bone marrow aspirate and trephine biopsy; bone marrow aspirates are frequently technically difficult due to marrow fibrosis and trephine biopsies are essential for the diagnosis of ML-DS.

Management of ML-DS

It is beyond the scope of this guideline to recommend treatment for ML-DS but modern reduced-anthracycline intensive chemotherapy regimens give long-term survival rates of 83–93% (Taga *et al*, 2016; Taub *et al*, 2017; Uffmann *et al*, 2017). In the UK, it is recommended that ML-DS be treated according to the CCLG ML-DS 2007 protocol (http://www.cclg.org.uk/).

Recommendations

- **Cases of TL-DS lacking LTS (see Table IV) should be monitored with FBC and liver function tests including conjugated bilirubin until there is spontaneous remission. In cases with persistent abnormalities in the FBC, *GATA1* mutation analysis can be considered. However, no value of monitoring for the persistence of the *GATA1* mutation and/or of quantitative assessment of the size of any residual *GATA1*-mutant clone in TL-DS or silent TL-DS has yet been demonstrated. (Grade 2B).**
- **All children with previous TL-DS or silent TL-DS should be monitored for progression to ML-DS with 3 monthly clinical review and FBC and film until the age of 2 years. If the FBC and film are normal and there are no clinical features of ML-DS, monitoring should continue 6 monthly until the age of 4 years. Abnormal blood counts should prompt early bone marrow aspirate and trephine biopsy (Grade 2B).**
- **ML-DS should be managed according to current national guidelines (Grade 1A).**

Fetal TL-DS

Despite arising *in utero*, it is uncommon for TL-DS to present before birth; <5% of neonatal cases have already been diagnosed antenatally. Typically, fetal TL-DS is detected on ultrasound scanning in the third trimester with hepatomegaly or splenomegaly (80%), hydrops fetalis (31%), pericardial

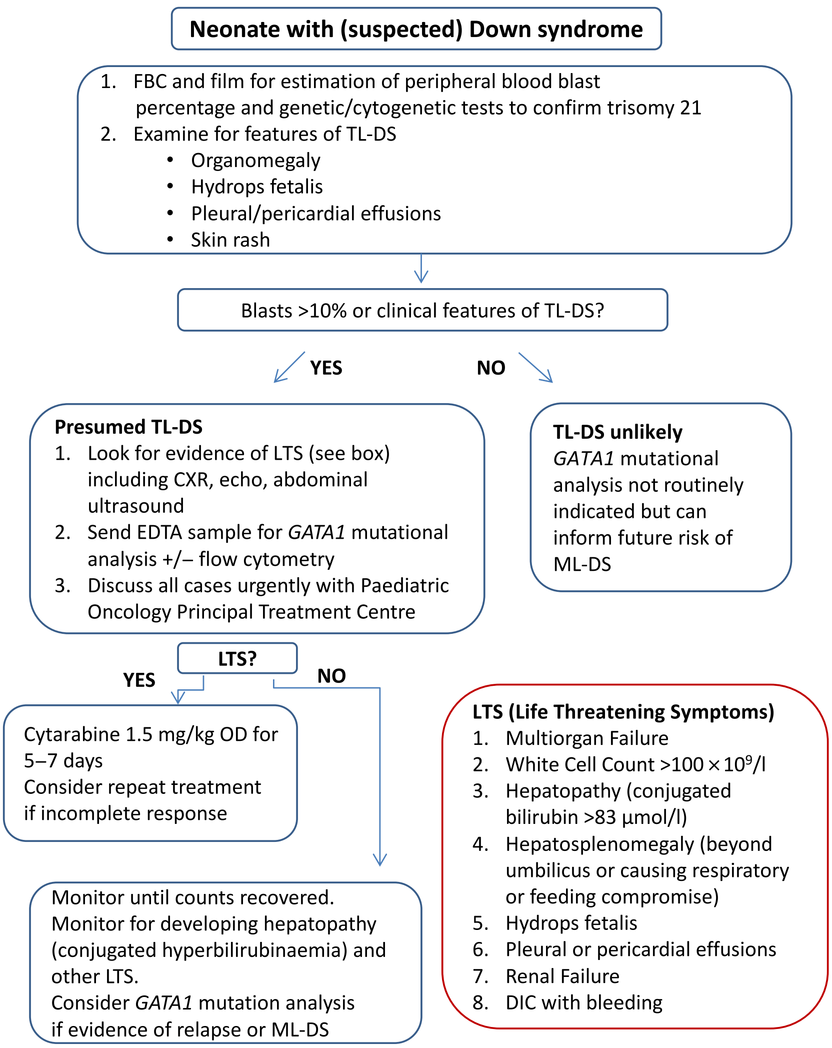

Fig 2. Investigation and Management of TL-DS. Summary algorithm showing the main recommended steps in the diagnosis and management of a child with TL-DS or suspected TL-DS. CXR: chest X-ray; FBC: full blood count; ML-DS: acute myeloid leukaemia of Down syndrome; OD: once daily; TL-DS: transient leukaemia of Down syndrome

effusion (23%), aberrant liquor volume (15%), cardiac abnormalities (12·8%), fetal ascites (10%), pleural effusion (8%) and peripheral oedema (3%). When fetal blood sampling is performed, blood films show leucocytosis with prominent blasts (96%), thrombocytopenia (86%) and abnormal liver function (92%) (Tamblyn *et al*, 2016).

Of 39 cases identified in the literature, only 14 (39%) were alive at follow up: there were two terminations of pregnancy, 12 intra-uterine deaths and 11 deaths in infancy, four in the first month. 15/23 cases were delivered before 37 weeks gestation.

With so few cases identified, all reported in ones and twos, there is only anecdotal evidence on which to base suggestions on management. Therapeutic interventions have included pericardiocentesis and intrauterine transfusion of packed red cells and platelets. Red cell transfusion might be useful for fetal anaemia but may risk hyperviscosity or hyperleucocytosis (Malin *et al*, 2010; Sukur *et al*, 2011; Tamblyn *et al*, 2016).

There are no cases reported of intrauterine therapy with cytarabine and it is important to note that spontaneous improvement is reported (Tamblyn *et al*, 2016). See Fig 2 for a summary of the recommended approach to investigation and management of TL-DS.

Recommendation

- **Where clinical features on fetal ultrasound scanning suggest TL-DS, fetal blood sampling with a FBC, blood film, liver function tests and *GATA1* mutation analysis should be performed to confirm the diagnosis. The poor outcome from retrospectively diagnosed fetal cases suggests that prompt, definitive diagnosis followed by close, multidisciplinary management of the pregnancy (fetal medicine specialist, neonatologist and paediatric haematologist) is likely to increase the chance of a better outcome through timing of delivery and judicious use of blood product support (Grade 2C).**

Disclaimer

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Declaration of interests

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Review process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH Guidelines website. If minor changes are required due to changes in level of evidence or significant additional evidence supporting current recommendations a new version of the current guidance will be issued on the BSH Guidelines website.

Audit tool

An audit template is available for this guideline and available on the following page of the BSH website:

http://www.b-s-h.org.uk/guidelines/?category=General+Haematology&p = 1&search=#guideline-filters__select__status

Author contributions

All of the authors reviewed the literature and contributed to the drafting and editing of the manuscript.

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