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β-Thalassemia intermedia: morbidity uncovered

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Asymptomatic Brain Magnetic Resonance Imaging Abnormalities In Splenectomized Adults With Thalassemia Intermedia

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Asymptomatic brain magnetic resonance imaging abnormalities in splenectomized adults with thalassemia intermedia

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Summary. *Background*: A high incidence of thrombotic events in thalassemia intermedia (TI) patients led to the identification of a hypercoagulable state. Brain involvement has not been widely studied in TI, although limited reports confirm a low incidence of overt stroke and high incidence of silent brain infarcts. *Patients/methods*: This was a cross-sectional study conducted on 30 adult, splenectomized TI patients. Patients were screenedforabsence of neurological signs orsymptoms, and stroke-related risk factors. Patient charts were reviewed for demographics, duration since splenectomy, and any history oftransfusion therapy. Blood samples were obtained for complete blood counts and serum ferritin. Direct determination of liver iron concentration (LIC) was performed by R2 magnetic resonance imaging(MRI). Brain MRI was performed on all patients, looking for ischemic lesions and/or atrophy. *Results*: The mean age of patients was 32.1 ± 11 years (range, 18–54 years), with a male to female ratio of 13:17. Eighteen patients (60%) had evidence of one or more white matter lesions (WMLs) on brain MRI, all involving the subcortical white matter. Fourteen patients had evidence of multiple WMLs, with a mean of 5 ± 10 lesions (range, 2 to > 40 lesions). The vast majority of patients (94%) had small (< 0.5 cm) to medium (0.5–1.5cm) WMLs, with only one patient showing evidence of a large (> 1.5 cm) WML. Eleven patients (37%) had mild cerebral atrophy. On multivariate analysisonly age and transfusion history were independently and significantly associated with the occurrence of zero, single or multiple WMLs. *Conclusion*: WMLs and brain atrophy are a common finding in adult, splenectomized, TI patients. Increasing age and transfusion naivety are associated with a higher incidence and multiplicity of lesions.

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Introduction

A hypercoagulable state in the thalassemia syndromes has been established [1]. This is attributed to a number of factors, including the procoagulant activity of damaged circulating red blood cells (RBCs), co-inheritance of coagulation defects, depletion of antithrombotic factors, endothelial inflammation and conditions that increase thrombotic burden [1]. The largest clinical study to date, on 8860 thalassemia patients, demonstrated that thromboembolic events (TEE) occur 4.38 times more frequently in thalassemia intermedia (TI) than thalassemia major (TM) patients [2]. In this and most other studies on TI patients, older age and splenectomy are implicated as significant risk factors for the development of TEE [2,3]. Brain involvement has not been widely studied and the incidence of clinically overt stroke in TI has not been established, although limited reports confirm lower incidence than TM [2]. However, in a study done to assess the rate of brain damage in patients with benign hemoglobinopathies, 37.5% of patients with TI showed asymptomatic brain damage on magnetic resonance imaging (MRI) [4], indicating that further investigation is warranted. This study aims to evaluate the incidence of and risk factors for silent brain abnormality in splenectomized adults with TI utilizing brain MRI.

Patients/methods

This was a cross-sectional studyconducted on all splenectomized TI patientsaged 18 years or older (n = 43) attending the Chronic Care Center (Lebanon) between June and December 2008. Afterscreening patients for exclusion criteria (Table 1), 30 patients were recruited into the study.

Table 1 Exclusion criteria

Criterion	Definition and assessment method
Neurological and/or gross cognitive signs or symptoms	Abnormality detected during medical history taking, neurological exam, or mini mental status exam (MMSE) performed by a qualified neurologist (AB)
Use of anticoagulant or antiplatelet therapy	Any current or previous history of anticoagulant or antiplatelet therapy
Diabetes	Use of antidiabetic drugs or a fasting blood sugar ≥ 126 mg dL^{-1}
Hypertension	Use of antihypertensive drugs or a blood pressure ≥ 140/90 mmHg on two readings 6 weeks apart
Cardiac disease	Any abnormality on electrocardiography or echocardiography including: arrhythmias, valvular disease, dysfunction, presence of atrial or ventricular thrombi, or pulmonary hypertension
Carotid stenosis	Evidence of > 50% narrowing of the carotid(s) on color-flow duplex scanning
Thrombophilia	Evidence of factor V Leiden, prothrombin or MTHFR mutations on genetic studies; or abnormality in protein C, protein S, antithrombin III, Lupus anticoagulant, or cardiolipin antibodies levels.
Smoking	Any current or previous history of smoking

MMSE, mini mental status exam; MTHFR, methyletetrahydrofolate reductase.

Brain MRIs were performed on a 3.0 Tesla, eight channel head coil, Achieva Philips Scanner using axial T1-weighted images (TR/TE, 450/10), T2 gradient-echo weighted images (TR/TE, 731/16), fluid-attenuated inversion recovery (FLAIR) images (TR/TE, 11000/125) and diffusion weighted imaging (TR/TE, 2312/68). Coronal FLAIR images (TR/TE, 11000/125) as well as coronal and sagittal T2 weighted images (TR/TE, 3000/80) were also obtained. No contrast material was administered. Two blinded neuroradiologists reviewed the studies, looking for ischemic lesions and/or atrophy. Infarction or ischemic lesions were defined as areas of abnormally increased signal intensity on the T2 and FLAIR weighted sequences and were classified by anatomic location and size. The size of lesions was classified into small (< 0.5 cm), medium (0.5–1.5 cm) and large (> 1.5 cm). For patients with multiple lesions, the largest lesion was used to define size. Atrophy was visually assessed as a decrease in brain volume greater than that which would be expected in a healthy volunteer of similar age and graded as mild, moderate or severe. This study was approved by the Institutional Review Board and written consents were obtained from all patients.

Patient charts were reviewed for demographics (age and gender), duration since splenectomy, and any history of transfusion therapy. Blood samples were obtained for assessment of total and fetal hemoglobin levels, nucleated RBC counts, platelet counts, and steady-state serum ferritin levels. Direct determination of liver iron concentration (LIC) was performed by R2 MRI using established methodology [5].

Statistical analysis

Descriptive statistics are expressed as means ± standard deviation (SD) or percentages where appropriate. Bivariate correlations between MRI findings and multiple variables were performed using independent-samples *t*-test for continuous variables and chi-square test for categorical variables. Multivariate analysis was conducted for all significant associations at the bivariate level using a generalized linear model. All *P*-values are two sided with the level of significance set at < 0.05.

Results

A total of 30 patients were included in this study (Table 2). Eighteen patients (60%) had evidence of one or more white

Table 2 Patients' characteristics

Parameter	Value
Mean age ± SD, years (range)	32.1 ± 11 (18–54)
Male:female	13:17
Mean duration since splenectomy ± SD, years (range)	17 ± 9.9 (2–36)
Transfusion history, *n* (%)	
None	18 (60)
Occasional	12 (40)
Mean Hb ± SD, g dL^{-1} (range)	8.6 ± 2.1 (4.9–13.1)
Mean HbF ± SD, % (range)	56.1 ± 30.6 (10.5–98.8)
Mean nucleated RBC count ± SD, × 10^3 mm^{-3} (range)	367.4 ± 319.8 (0–1366)
Mean platelet count ± SD, × 10^3 mm^{-3} (range)	791.2 ± 355.3 (189–1602)
Mean serum ferritin ± SD, ng mL^{-1} (range)	1176 ± 641.9 (116–3158)
Mean LIC ± SD, mg Fe per g dw (range)	11.3 ± 7.8 (1–32.1)

Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell; LIC, liver iron concentration; dw, dry weight.

Table 3 Distribution of number, location and size of identified white matter lesions (WMLs) on brain MRI

Parameter	*n* (%)
Number	
Single	4 (22.2)
Multiple	14 (77.8)
Location	
Frontal	17 (94.4)
Parietal	9 (50)
Temporal	1 (5.6)
Occipital	3 (16.7)
Internal capsule	1 (5.6)
External capsule	5 (27.8)
Size*	
Small (< 0.5 cm)	10 (55.5)
Medium (0.5–1.5 cm)	7 (38.9)
Large (> 1.5 cm)†	1 (5.6)

*For patients with multiple lesions, the largest lesion was used to define size. †The possibility of misreading confluent multiple lesions was excluded radiologically based on lesion shape.

matter lesions (WMLs) on brain MRI, all involving the subcortical white matter (Table 3). Most of those patients (14 patients) had evidence of multiple WMLs, with a mean of 5 ± 10 lesions (range, 2 to > 40 lesions). The frontal subcortical white matter was nearly always involved, followed by the parietal and occipital subcortical white matter. The external capsule was involved in 28% of patients. The vast majority of patients (94%) had evidence of small to medium WMLs, with only one patient showing evidence of a large WML (Fig. 1). Eleven patients (37%) had evidence of mild cerebral atrophy, 10 of whom had associated WMLs.

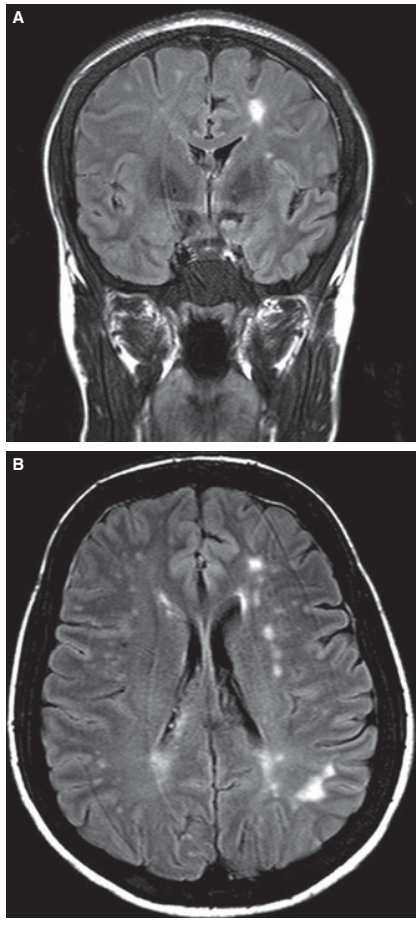

Fig. 1. (A) Coronal and (B) axial FLAIR images showing multiple foci of high signal seen in the subcortical and periventricular white matter with one large lesion (1.7 cm) seen in the left parietal white matter.

Risk factors for white matter lesions

Among all study variables, only age (*t*-test) and transfusion history (chi-square test) were significantly associated with presence of WMLs (Table 4). The mean age was higher in patients who had white matter lesions compared with those who had no lesions (mean age difference of 10 years). A logistic regression analysis to determine the probability of having a WML [Y] at a particular age was performed (Fig. 2) with the following result:

$$[\mathrm{Y}] = \exp(-2.9179 + (0.107846)^{*}\mathrm{age}/1 + \exp(-2.9179 + (0.107846)^{*}\mathrm{age})).$$

This formula had a predictive value of 72.4%.

In addition, in the subgroup of patients with WMLs the mean age of patients who had multiple lesions was higher than those with single lesions (37.1 vs. 30.7 years); although this difference did not reach statistical significance ($P = 0.342$).

Patients who occasionally received transfusions had a significantly lower incidence of WMLs (25%) compared with those who had never received a transfusion (83.3%) ($P = 0.001$). When WMLs were present, patients who were occasionally transfused had a significantly lower incidence of multiple lesions (40%) compared with patients who had never been transfused (92.3%) ($P = 0.017$).

Other variables, including gender, and means of duration since splenectomy, Hb, HbF, nucleated RBC count, platelet count, serum ferritin and LIC, were not significantly associated with the presence or number of WMLs.

On multivariate analysis using a generalized linear model, both age ($P = 0.018$) and transfusion history ($P = 0.008$)

Table 4 Comparison of study variables between patients with and without brain white matter lesions (WMLs)

Parameter	WML neg $n = 12$	WML pos $n = 18$	*P*-value
Mean age (years)	26.1	36.1	**0.011**
Male:female	9:3	8:10	0.098
Mean duration since splenectomy (years)	12.3	18.3	0.156
Transfusion history, *n* (%)			
None	3 (25)	15 (83.3)	**0.001**
Occasional	9 (75)	3 (16.7)	
Mean Hb (g dL^{-1})	7.9	8.8	0.230
Mean HbF (%)	44.8	63.7	0.136
Mean nucleated RBC count ($\times 10^3$ mm^{-3})	413.8	332.7	0.517
Mean platelet count ($\times 10^3$ mm^{-3})	862.7	737.5	0.366
Mean serum ferritin (ng mL^{-1})	1083	1296	0.396
Mean LIC (mg Fe per g dw)	10.3	11.9	0.052

WML, white matter lesion; MRI, magnetic resonance imaging; Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell; LIC, liver iron concentration; dw, dry weight. Bold values are those that are statistically significant ($P < 0.05$).

Fig. 2. Logistic regression analysis for the probability of having white matter lesions (WMLs) with increasing age.

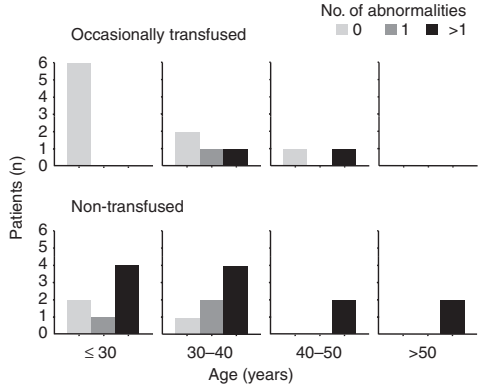

Fig. 3. A histogram showing the number of patients having no, single or multiple white matter lesions (WMLs) according to age and transfusion history.

were independently and significantly associated with the occurrence of zero, single or multiple WMLs (Fig. 3). Among all study variables, only age was significantly associated with presence or absence of brain atrophy. The mean age was higher in patients with (38.5 years) than in those without (28.3 years) brain atrophy ($P = 0.011$).

Discussion

In our study, brain MRI evaluation demonstrates that WMLs and brain atrophy are a common finding in adult, splenectomized, TI patients. Moreover, increasing age and transfusion naivety are associated with a higher incidence and multiplicity of lesions. Our study echoes that of Manfre *et al.* [4] on 16 TI patients, and further brings attention to this previously uninvestigated complication in TI patients. The higher incidence (60% vs. 37%) and multiplicity (77.8% vs. 12.5%) of WMLs in our cohort compared with that of Manfre's may be attributed to higher patient age (mean age 32.1 vs. 29 years) and imaging modality used (3.0 T-MRI vs. 0.5 T-MRI).

Although neither our study nor that of Manfre *et al.* [4] had a control group, the incidence of WMLs in the aging brain of healthy volunteers has been investigated. High incidence of WMLs among healthy elderly populations (e.g. those aged greater than 70) has been reported in many studies [6–11]. However, relatively fewer brain MRI studies recruited populations (or samples) of healthy individuals < 50 years of age (Table 5) [12–18]. The outcome of these studies reveals that the frequency of WMLs and/or cerebral atrophy in healthy young individuals ranges from 0% to 11%. Comparing this figure with the frequency observed in our patients (60%), it seems more likely that the changes described in this report are pathological rather than normal variations. This observation is similar to that reported in patients with sickle cell disease (SCD), where incidence of silent brain ischemia has been well studied and reports document occurrences up to 83% [19].

The presence of WMLs raises the suspicion of several underlying diagnoses. However, in our patients these diagnoses may be ruled out based on the radiological appearance of lesions (e.g. viral encephalopathy or multiple sclerosis) or absence of associated risk factors and clinical symptoms (e.g. vasculitis or Binswanger). Thus, MRI findings in this report most likely represent ischemic lesions. WMLs correspond to increasing severity of ischemic tissue damage, ranging from mild perivascular alterations to large areas with variable loss of fibers, multiple small cavitations, and marked arteriolosclerosis. Microcystic infarcts and patchy rarefaction of myelin are also characteristics of irregular periventricular high signal intensities [20]. These lesions were significantly associated with impaired cognitive skills, suggesting they can be nearly as damaging to cognitive function as overt stroke [21]. In addition to cognitive effects, WMLs have a role in the decline of other functional performances, and this places individuals with higher-grade lesions at increased risk of developing disability [21]. Although patients in this report had no evidence of gross cognitive disabilities, minor and more specific cognitive disability or psychological disease cannot be ruled out.

Table 5 Summary of brain MRI studies that recruited a population (or sample) of healthy individuals < 50 years of age

Reference	*n*	Mean age* (range), years	Abnormality	Frequency (%)
Fazekas 1989 [12]	87	(31–83)	WML	11% for patients in 4th decade
Salonen 1997 [13]	23	(30–53)	WML	0
Katzman 1999 [14]	1000	30.6 (3–83)	WML	0.8
Hopkins 2006 [15]	243	(16–65)	WML	5.3%
Weber 2006 [16]	750	(45–59)	WML	7.2%
Vernooij 2007 [17]	2536	20.5 (17–35)	Atrophy	0.43
Yamada 2008 [18]	16 206	70 (39–90)	WML	0.37

WML, white matter lesions > 0.5 mm. *Where available.

The anemia in TI does not seem to contribute to the pathogenesis of WMLs, as in our analysis there was no statistically significant difference in mean hemoglobin level of patients with and without WMLs. The literature only supports a role for acute anemia in cerebral injury of perioperative and critical care patients [22,23], increased morbidity and mortality in patients with acute anemia and first-ever stroke [24], and a potential role for chronic anemia in cerebral injury of patients with multiple stroke-related risk factors [25]. Thus, it seems less likely that chronic anemia in our patients caused WMLs. However, the hemolytic anemia implicated in patients with TI causes iron-dependent oxidation of membrane proteins and formation of red-cell 'senescence' antigens such as phosphatidylserine that cause thalassemic red cells to be rigid and deformed and to aggregate, resulting in premature cell removal. Studies have shown that, as such, thalassemic RBCs may be a source of negatively charged phospholipids, which can eventually increase thrombin generation [26–32]. These abnormalities have been reduced to normal range after the patients have received a blood transfusion, which decreases the number of circulating damaged RBCs [33]. This could partly explain why patients in our study who were never transfused had a higher incidence of WMLs than patients receiving occasional transfusions. As such, several trials in patients with SCD demonstrated a significantly reduced risk of silent strokes in participants receiving blood transfusions [34,35]. This should sound promising for patients with TI; however, it should be balanced against the iron loading secondary to chronic transfusions, especially in this patient population with high susceptibility to iron overload secondary to endogenously increased intestinal iron absorption [36].

Clinical observations have suggested that splenectomy in TI can contribute to an increased susceptibility to thrombosis [2,3]. The development of these complications has been ascribed to the presence of high platelet counts following splenectomy [37,38] and/or to increased number of nucleated RBCs [39]. In splenectomized TI patients, thrombin generation was significantly higher than in control subjects and patients who had not undergone splenectomy [3]. The high incidence of ischemic lesions in our splenectomized population may be attributable to such pathophysiology. The contribution of splenectomy to hypercoagulability in other hemolytic anemias (excluding SCD where vasculopathy and sickling dominate the picture, and TM where the presence of co-morbidities defines the risk of stroke) may warrant similar brain MRI evaluation, as cerebrovascular events are increasingly being reported [40,41]. However, the lack of association between the duration since splenectomy, nucleated RBC levels, platelet counts and the incidence of brain pathology questions the clinical implication of the proposed mechanism.

The milder course of TI compared with TM has provided patients with survival benefit. However, this increased life expectancy is not without its own side-effects. Our study further adds to the role of age in accumulating complications in TI patients by demonstrating that both WMLs and atrophy may eventually ensue. This brings further attention to our aging TI patient population. It directly calls for earlier intervention to prevent serious long-term sequelae and fortifies the notion that thalassemia is transforming into an adult disease [42]. If the pathogenesis of these lesions is to be understood and potentially other modifiable factors to be identified, the study of individuals in the earliest stages of development of the lesions would be valuable.

Further research is required to examine the use of transfusion in preventing brain ischemia in adult, splenectomized, TI patients. The need to delay or halt the progression of WMLs should lead to further clarification of the role of some risk factors and performance of therapeutic trials where WMLs are used as a surrogate marker for the endpoint of small-vessel disease.

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Disclosure of Conflict of Interests

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References

1 Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. *Blood Rev* 2008; **22**: 283–92.

2 Taher A, Isma'eel H, Mehio G, Bignamini D, Kattamis A, Rachmilewitz EA, Cappellini MD. Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and Iran. *Thromb Haemost* 2006; **96**: 488–91.

3 Cappellini MD, Robbiolo L, Bottasso BM, Coppola R, Fiorelli AP, Mannucci AP. Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. *Br J Haematol* 2000; **111**: 467–73.

4 Manfre L, Giarratano E, Maggio A, Banco A, Vaccaro G, Lagalla R. MR imaging of the brain: findings in asymptomatic patients with thalassemia intermedia and sickle cell-thalassemia disease. *AJR Am J Roentgenol* 1999; **173**: 1477–80.

5 St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E, Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood* 2005; **105**: 855–61.

6 Enzinger C, Smith S, Fazekas F, Drevin G, Ropele S, Nichols T, Behrens T, Schmidt R, Matthews PM. Lesion probability maps of white matter hyperintensities in elderly individuals: results of the Austrian stroke prevention study. *J Neurol* 2006; **253**: 1064–70.

7 Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. *Lancet* 2003; **361**: 2046–8.

8 de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gjin J, Breteler MM. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* 2001; **70**: 9–14.

9 Wen W, Sachdev PS. Extent and distribution of white matter hyperintensities in stroke patients: the Sydney Stroke Study. *Stroke* 2004; **35**: 2813–9.

10 Longstreth WT Jr, Manolio TA, Arnold A, Burke GL, Bryan N, Jungreis CA, Enright PL, O'Leary D, Fried L. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. *Stroke* 1996; **27**: 1274–82.

11 Ylikoski R, Ylikoski A, Erkinjuntti T, Sulkava R, Raininko R, Tilvis R. White matter changes in healthy elderly persons correlate with attention and speed of mental processing. *Arch Neurol* 1993; **50**: 818–24.

12 Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur Neurol* 1989; **29**: 164–8.

13 Salonen O, Autti T, Raininko R, Ylikoski A, Erkinjuntti T. MRI of the brain in neurologically healthy middle-aged and elderly individuals. *Neuroradiology* 1997; **39**: 537–45.

14 Katzman GL, Dagher AP, Patronas NJ. Incidental findings on brain magnetic resonance imaging from 1000 asymptomatic volunteers. *JAMA* 1999; **282**: 36–9.

15 Hopkins RO, Beck CJ, Burnett DL, Weaver LK, Victoroff J, Bigler ED. Prevalence of white matter hyperintensities in a young healthy population. *J Neuroimaging* 2006; **16**: 243–51.

16 Weber F, Knopf H. Incidental findings in magnetic resonance imaging of the brains of healthy young men. *J Neurol Sci* 2006; **240**: 81–4.

17 Vernooij MW, Ikram MA, Tanghe HL, Vincent AJ, Hofman A, Krestin GP, Niessen WJ, Breteler MM, van der Lugt A. Incidental findings on brain MRI in the general population. *N Engl J Med* 2007; **357**: 1821–8.

18 Yamada K, Nagakane Y, Sasajima H, Nakagawa M, Mineura K, Masunami T, Akazawa K, Nishimura T. Incidental acute infarcts identified on diffusion-weighted images: a university hospital-based study. *AJNR Am J Neuroradiol* 2008; **29**: 937–40.

19 Powars DR, Conti PS, Wong WY, Groncy P, Hyman C, Smith E, Ewing N, Keenan RN, Zee CS, Harold Y, Hiti AL, Teng EL, Chan LS. Cerebral vasculopathy in sickle cell anemia: diagnostic contribution of positron emission tomography. *Blood* 1999; **93**: 71–9.

20 Wen W, Sachdev PS, Li JJ, Chen X, Anstey KJ. White matter hyperintensities in the forties: their prevalence and topography in an epidemiological sample aged 44-48. *Hum Brain Mapp* 2009; **30**: 1155–67.

21 Pantoni L, Poggesi A, Inzitari D. The relation between white-matter lesions and cognition. *Curr Opin Neurol* 2007; **20**: 390–7.

22 Hare GM, Tsui AK, McLaren AT, Ragoonanan TE, Yu J, Mazer CD. Anemia and cerebral outcomes: many questions, fewer answers. *Anesth Analg* 2008; **107**: 1356–70.

23 Kramer AH, Zygun DA. Anemia and red blood cell transfusion in neurocritical care. *Crit Care* 2009; **13**: R89.

24 Huang WY, Chen IC, Meng L, Weng WC, Peng TI. The influence of anemia on clinical presentation and outcome of patients with first-ever atherosclerosis-related ischemic stroke. *J Clin Neurosci* 2009; **16**: 645–9.

25 Warnock DG, McClellan W, McClure LA, Newsome B, Campbell RC, Audhya P, Cushman M, Howard VJ, Howard G. Prevalence of chronic kidney disease and anemia among participants in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Cohort Study: baseline results. *Kidney Int* 2005; **68**: 1427–31.

26 Rund D, Rachmilewitz E. Beta-thalassemia. *New Engl J Med* 2005; **353**: 1135–46.

27 Shinar E, Rachmilewitz EA, Lux SE. Differing erythrocyte membrane skeletal protein defects in alpha and beta thalassemia. *J Clin Invest* 1989; **83**: 404–10.

28 Hershko C, Graham G, Bates GW, Rachmilewitz EA. Nonspecific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. *Br J Haematol* 1978; **40**: 255–63.

29 Kuypers FA, de Jong K. The role of phosphatidylserine in recognition and removal of erythrocytes. *Cell Mol Biol (Noisy-le-grand)* 2004; **50**: 147–58.

30 Tavazzi D, Duca L, Graziadei G, Comino A, Fiorelli G, Cappellini MD. Membrane-bound iron contributes to oxidative damage of beta-thalassaemia intermedia erythrocytes. *Br J Haematol* 2001; **112**: 48–50.

31 Borenstain-Ben Yashar V, Barenholz Y, Hy-Am E, Rachmilewitz EA, Eldor A. Phosphatidylserine in the outer leaflet of red blood cells from beta-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. *Am J Hematol* 1993; **44**: 63–5.

32 Helley D, Eldor A, Girot R, Ducrocq R, Guillin MC, Bezeaud A. Increased procoagulant activity of red blood cells from patients with homozygous sickle cell disease and betathalassemia. *Thromb Haemost* 1996; **76**: 322–7.

33 Chen S, Eldor A, Barshtein G, Zhang S, Goldfarb A, Rachmilewitz E, Yedgar S. Enhanced aggregability of red blood cells of beta-thalassemia major patients. *Am J Physiol* 1996; **270**: H1951–6.

34 Brousse V, Hertz-Pannier L, Consigny Y, Bresson J, Girot R, Mirre E, Lenoir G, Montalembert M. Does regular blood transfusion prevent progression of cerebrovascular lesions in children with sickle cell disease? *Ann Hematol* 2009; **88**: 185–8.

35 Hirst C, Wang WC. Blood transfusion for preventing stroke in people with sickle cell disease. *Cochrane Database Syst Rev* 2002; **1**: CD003146.

36 Origa R, Galanello R, Ganz T, Giagu N, Maccioni L, Faa G, Nemeth E. Liver iron concentrations and urinary hepcidin in b-thalassemia. *Haematologica* 2007; **92**: 583–8.

37 Cappellini MD, Grespi E, Cassinerio E, Bignamini D, Fiorelli G. Coagulation and splenectomy: an overview. *Ann N Y Acad Sci* 2005; **1054**: 317–24.

38 Atichartakarn V, Angchaisuksiri P, Aryurachai K, Chuncharunee S, Thakkinstian A. In vivo platelet activation and hyperaggregation in hemoglobin E/beta-thalassemia: a consequence of splenectomy. *Int J Hematol* 2003; **77**: 299–303.

39 Atichartakarn V, Angchaisuksiri P, Aryurachai K, Onpun S, Chuncharunee S, Thakkinstian A, Atamasirikul K. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/beta thalassaemic patients. *Br J Haematol* 2002; **118**: 893–8.

40 Audebert HJ, Planck J, Eisenburg M, Schrezenmeier H, Haberl RL. Cerebral ischemic infarction in paroxysmal nocturnal hemoglobinuria report of 2 cases and updated review of 7 previously published patients. *J Neurol* 2005; **252**: 1379–86.

41 Kim BJ, Park KW, Koh SB, Kim HK, Jung HL, Park MK, Lee DH. Stroke induced by splenectomy in hemoglobin Madrid: autopsy clues to the underlying mechanism. *Blood Coagul Fibrinolysis* 2005; **16**: 141–4.

42 Musallam K, Cappellini MD, Taher A. Challenges associated with prolonged survival of patients with thalassemia: transitioning from childhood to adulthood. *Pediatrics* 2008; **121**: e1426–9.