

acquired TTP but with an atypical presentation. A suboptimal response to PEX, defined as the absence of a steadily declining lactate dehydrogenase level and an increase in the platelet count after 4–5 days of daily PEX in the context of ADAMTS13 >10%, would lead us to consider therapy with eculizumab over intensified PEX or immune-based therapy as might be considered in TTP.

Acknowledgements

SRC and HMW designed the study, performed the research, analysed the data, and wrote the paper. SY performed the research and analysed the data.

Spero R. Cataland¹
Shangbin Yang²
Haifeng M. Wu²

¹Division of Hematology, Department of Internal Medicine, Ohio State University, Columbus, OH, USA and ²Department of Pathology, Ohio State University, Columbus, OH, USA
E-mail: spero.cataland@osumc.edu

Keywords: aHUS, TTP, ADAMTS13, platelet count, creatinine

First published online 1 February 2012
doi: 10.1111/j.1365-2141.2012.09032.x

References

- Bianchi, V., Robles, R., Alberio, L., Furlan, M. & Lammler, B. (2002) Von Willebrand factor-cleaving protease (ADAMTS13) in thrombotic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. *Blood*, **100**, 710–713.
- Coppo, P., Bengoufa, D., Veyradier, A., Wolf, M., Bussel, A., Millot, G.A., Malot, S., Heshmati, F., Mira, J.P., Boulanger, E., Galicier, L., Durey-Dragon, M.A., Fremaux-Bacchi, V., Ramakers, M., Pruna, A., Bordessoule, D., Gouilleux, V., Scrobohaci, M.L., Vernant, J.P., Moreau, D., Azoulay, E., Schlemmer, B., Guillevin, L. & Lascoued, K. (2004) Severe ADAMTS13 deficiency in adult idiopathic thrombotic microangiopathies defines a subset of patients characterized by various autoimmune manifestations, lower platelet count, and mild renal involvement. *Medicine (Baltimore)*, **83**, 233–244.
- Froehlich-Zahnd, R., George, J.N., Vesely, S.K., Terrell, D.R., Aboulatova, K., Dong, J.F., Luken, B.M., Voorberg, J., Budde, U., Sulzer, I., Lammler, B. & Kremer Hovinga, J.A. (2011) Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura. *Haematologica*. Epub ahead of print. doi: 10.3324/haematol.2011.051433.
- Hovinga, J.A., Vesely, S.K., Terrell, D.R., Lammler, B. & George, J.N. (2010) Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood*, **115**, 1500–1511; quiz 1662.
- Legendre, C.M., Babu, S., Furman, R.R., Sheerin, N.S., Cohen, D.J., Gaber, O., Eitner, F., Delmas, Y., Loirat, C., Greenbaum, L.A. & Zimhackl, L.B. (2010) Safety & efficacy of eculizumab in aHUS patients resistant to plasma therapy: interim analysis from a phase II trial. *Journal of the American Society of Nephrology*, **21**, 93A.
- Mache, C.J., Acham-Roschitz, B., Fremaux-Bacchi, V., Kirschfink, M., Zipfel, P.F., Roedel, S., Vester, U. & Ring, E. (2009) Complement inhibitor eculizumab in atypical hemolytic uremic syndrome. *Clin J Am Soc Nephrol*, **4**, 1312–1316.
- Remuzzi, G. (2003) Is ADAMTS-13 deficiency specific for thrombotic thrombocytopenic purpura? No. *J Thromb Haemost*, **1**, 632–634.
- Remuzzi, G., Galbusera, M., Noris, M., Canciani, M.T., Daina, E., Bresin, E., Contaretti, S., Caprioli, J., Gamba, S., Ruggerenti, P., Perico, N. & Mannucci, P.M. (2002) von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood*, **100**, 778–785.
- Salmon, J.E., Heuser, C., Triebwasser, M., Liszewski, M.K., Kavanagh, D., Roumenina, L., Branch, D.W., Goodship, T., Fremaux-Bacchi, V. & Atkinson, J.P. (2011) Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med*, **8**, e1001013.
- Tsai, H.M. (2003) Is severe deficiency of ADAMTS-13 specific for thrombotic thrombocytopenic purpura? Yes. *Journal of Thrombosis and Haemostasis*, **1**, 625–631.

Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the EIF6 gene and benign prognosis

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disease [Online Mendelian Inheritance in Man (OMIM) #260400] that is caused by mutations in the *SBDS* gene in at least 90% of cases (Dror, 2005). It is characterized by exocrine pancreatic insufficiency, skeletal abnormalities, and bone marrow (BM) failure with variable severity of neutropenia, thrombocytopenia, and anaemia, with a risk, as high as 30%, for developing myelodysplastic syndrome

(MDS) and/or acute myeloid leukaemia (AML) (Dror, 2005). Clonal chromosome changes, mainly involving chromosomes 7 and 20, are often found in the BM of SDS patients, the most frequent abnormalities being an isochromosome for the long arms of chromosome 7, i(7)(q10), and a deletion of the long arms of chromosome 20, which was shown to be an interstitial deletion, with breakpoints in the bands q11.21 and q13.32 (Maserati *et al*, 2011). The rela-

tionship between these and other chromosome changes in BM cells and the risk of MDS/AML is a subject for debate (Dror, 2005).

Some conclusions were drawn for the i(7)(q10), which has been associated with a lower risk and a more benign course (Shimamura, 2006). An intriguing explanation was given by the observation that the mutation of the *SBDS* gene duplicated in the long arms of chromosome 7 was consistently one of the two recurrent mutations, c.258+2T>C, which had been shown to allow the production of a small amount of the SBDS protein (Minelli *et al*, 2009).

Only one paper is available in the literature concerning the other more common anomaly, the interstitial deletion of most of the long arms of chromosome 20, int del (20)(q11.21q13.32): it provides convincing evidence of a benign course of SDS also in patients bearing this anomaly as an isolated change (Crescenzi *et al*, 2009). Nevertheless, some review papers have accepted the concept that the int del (20)(q11.21q13.32) is a good prognostic sign, as well as the i(7)(q10) (Shimamura, 2006).

We note that the i(7)(q10) as a clonal anomaly in BM is almost exclusive to SDS, whereas the deletion of the long arms of chromosome 20, which has not been investigated in more detail with recent techniques of molecular cytogenetics, is also a recurrent change in different myelodysplastic and myeloproliferative disorders: in particular, it has been associated with a relatively benign prognosis in MDS (Steensma & List, 2005), at least when present as the sole anomaly.

The int del (20)(q11.21q13.32) was found in 15 of the 67 SDS patients that we have followed since 1999. Some details on these patients were previously reported (Maserati *et al*, 2006, 2009). The follow-up period of these 15 patients varied from less than one year to 10 years: the percentage of abnormal cells in the BM was very small in some cases, and was often the case for patients in whom the anomaly was recently detected, with a shorter follow-up. To evaluate the possible prognostic value of the presence of the int del (20)(q11.21q13.32), we arbitrarily chose to take into account only the six cases with an abnormal clone representing more than 10% of BM cells (range 10.6–90%). None of these patients progressed to MDS/AML, while the BM and peripheral blood conditions remained almost stable in all cases, without any progression to severe BM aplasia. One patient died, but this occurred after heart transplantation due to dilative cardiomyopathy. So, despite the limit of the small number of patients, the int del (20)(q11.21q13.32) could be confirmed as a benign change also in our SDS cohort.

Investigating the function of the SBDS protein in ribosome biogenesis, Finch *et al* (2011) showed that it couples with the GTPase EFL1 to cause the release of the EIF6 protein from the pre-60S ribosome subunit, thus permitting its binding with the 40S subunit and allowing the formation of actively translating 80S ribosome. So, SDS would be

caused by the defective presence of the SBDS protein and the subsequent impairment of this mechanism. The gene coding for EIF6 is located on chromosome 20, in the band q11.22 (bp 33,330,139–33,336,008), within the segment of the long arms lost in the int del (20)(q11.21q13.32). This was the case for the 14 patients in our series who carried the recurrent broader interstitial deletion, int del (20)(q11.21q13.32), but *EIF6* was lacking also in one patient who presented with a tiny interstitial deletion, different from the more common anomaly [identified as unique patient number (UPN) 14 in Maserati *et al*, 2006]: the deletion led in this case to the loss of the region q11.21–q11.23, as ascertained by fluorescent *in situ* hybridization (FISH) and microarray-based comparative genomic hybridization (a-CGH). FISH also confirmed the loss of the *EIF6* gene on BM mitoses and interphase nuclei of three of our patients with the probe CTD 2559C9 (Invitrogen Corporation, Carlsbad, CA, USA), which recognizes a sequence including the entire *EIF6* gene. The results showed only one signal in 5/7 mitoses and 184/366 nuclei in patient UPN 13 (Maserati *et al*, 2006), in 36/55 mitoses in UPN 17, and in 147/229 nuclei in UPN 20 (Maserati *et al*, 2009).

These evidences lead us to postulate that the benign prognosis of the SDS patients with the int del (20)(q11.21q13.32) is due to a gene/dosage effect for the EIF6 protein in the cells of the abnormal clone in the BM: the decreased amount of EIF6 would facilitate ribosome biogenesis in these cells, with some sort of rescue mechanism primed by the acquired loss of chromosome material.

Acknowledgements

This work was supported by grants from Associazione Italiana Sindrome di Shwachman (AISS) and Fondazione Banca del Monte di Lombardia.

Barbara Pressato
Roberto Valli
Cristina Marletta
Lydia Mare
Giuseppe Montalbano
Francesco Lo Curto
Francesco Pasquali
Emanuela Maserati

Genetica umana e medica, Dipartimento di Medicina Clinica e Sperimentale, Università dell'Insubria, Varese, Italy.
E-mail: emanuela.maserati@uninsubria.it

Keywords: Shwachman-Diamond syndrome, del(20), EIF6 gene

First published online 1 February 2012
 doi: 10.1111/j.1365-2141.2012.09033.x

References

- Crescenzi, B., La Starza, R., Sambani, C., Parchari-dou, A., Pierini, V., Nofrini, V., Brandimarte, L., Matteucci, C., Aversa, F., Martelli, M.F. & Mecucci, C. (2009) Totipotent stem cells bearing del (20q) maintain multipotential differentiation in Shwachman Diamond syndrome. *British Journal of Haematology*, **144**, 116–119.
- Dror, Y. (2005) Shwachman-Diamond syndrome. *Pediatric Blood Cancer*, **45**, 892–901.
- Finch, A.J., Hilcenko, C., Basse, N., Drynan, L.F., Goyenechea, B., Menne, T.F., González Fernández, A., Simpson, P., D' Santos, C.S., Arends, M.J., Donadieu, J., Bellanné-Chantelot, C., Costanzo, M., Boone, C., McKenzie, A.N., Freund, S.M.V. & Warren, A. (2011) Uncoupling of GTP hydrolysis from eIF6 release on the ribosome causes Shwachman-Diamond syndrome. *Genes and Development*, **25**, 917–929.
- Maserati, E., Minelli, A., Pressato, B., Valli, R., Crescenzi, B., Stefanelli, M., Menna, G., Sainati, L., Poli, F., Panarello, C., Zecca, M., Lo Curto, F., Mecucci, C., Danesino, C. & Pasquali, F. (2006) Shwachman syndrome as mutator phenotype responsible for myeloid displasia/neoplasia through karyotype instability and chromosome 7 and 20 anomalies. *Genes Chromosomes and Cancer*, **45**, 375–382.
- Maserati, E., Pressato, B., Valli, R., Minelli, A., Sainati, L., Patitucci, F., Marletta, C., Maseronuzzi, A., Poli, F., Lo Curto, F., Locatelli, F., Danesino, C. & Pasquali, F. (2009) The route to development of myelodysplastic syndrome/acute myeloid leukaemia in Shwachman-Diamond syndrome: the role of ageing, karyotype instability, and acquired chromosome anomalies. *British Journal of Haematology*, **145**, 190–197.
- Maserati, E., Pressato, B., Marletta, C., Valli, R., Mare, L., Montalbano, G., Lo Curto, F. & Pasquali, F. (2011) The use of microarray-based comparative genomic hybridization (a-CGH) to detect and define chromosome imbalances in the bone marrow of SDS patients. 6th International Congress on Shwachman-Diamond Syndrome, New York, NY, June 28–30, 2011, Abstract 13. The New York Academy of Sciences, New York. <http://www.nyas.org/asset.axd?id=f5797b54-762d-45dc-80a7-6484c4e018aa&t=634460867330870000>.
- Minelli, A., Maserati, E., Nicolis, E., Zecca, M., Sainati, L., Longoni, D., Lo Curto, F., Menna, G., Poli, F., De Paoli, E., Cipolli, M., Locatelli, F., Pasquali, F. & Danesino, C. (2009) The iso-chromosome i(7)(q10) carrying c.258+2t>c mutation of the SBDS gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia*, **23**, 708–711.
- Shimamura, A. (2006) Shwachman-Diamond syndrome. *Seminars in Hematology*, **43**, 178–188.
- Steenma, D.P. & List, A.F. (2005) Genetic testing in the myelodysplastic syndromes: molecular insights into hematologic diversity. *Mayo Clinic Proceedings*, **80**, 681–698.

Safety of deferasirox in sickle cell disease patients with co-existing liver impairment

We read with great interest the article by Vichinsky *et al* (2011) regarding the long-term safety and efficacy of deferasirox (Exjade[®], Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA) in transfusional iron-overloaded patients with sickle cell disease (SCD).

In the initial study, deferasirox use had been associated with reversible increases in liver function tests (LFTs) in a relatively small percentage of patients (3.8%) (Vichinsky *et al*, 2007). Only one patient in this study was confirmed (with deferasirox re-challenge) to have LFT elevations caused by deferasirox. In the follow-up study three deaths were reported (Vichinsky *et al*, 2011), one of them in a patient with underlying liver disease secondary to hepatitis C virus (HCV) infection, in addition to haemosiderosis. This patient had presented with hepatitis C and abnormal LFTs at the time of enrolment and was diagnosed with hepatic failure following 40 months of treatment with deferasirox. According to the investigators' judgment, hepatic failure was attributed to the underlying liver disease and not to the study drug. In light of the above mentioned LFT elevations associated with the use of deferasirox in the initial report, we believe that Vichinsky *et al* (2011) should have provided more detailed information on the course of this patient to support their contention. The stage of liver disease at the time of enrollment, as well as the virological status of the patient are

of considerable significance in order to fully understand this dismal outcome. Advanced fibrosis or 'active' viral hepatitis, if present, could have been implicated in this patient's liver failure. However, patients with 'active' hepatitis C (patients with positive HCV RNA) were excluded from these two studies as per exclusion criteria (Vichinsky *et al*, 2007, 2011). Of note, patients with negative HCV RNA are currently considered to be cured, as the reappearance of HCV RNA following successful antiviral treatment is seen in less than 1% of cases (Swain *et al*, 2010). Furthermore, transmission of HCV is currently unusual, especially in the setting of a well-structured clinical study.

In an effort to expand our knowledge on deferasirox safety in patients with liver disease we have published our anecdotal experience of patients with chronic hepatitis C receiving iron chelation treatment with deferasirox (Paschos *et al*, 2011). This included three adults with β -thalassaemia major and 'active' hepatitis C. Two of them received deferasirox and peg-interferon in combination with ribavirin, whereas the other patient only received deferasirox and refused HCV treatment. No significant or unusual adverse events were observed with the use of deferasirox in this subset of patients with liver disease. Of note, viral response during therapy was achieved in the two patients that had received antiviral treatment.