Italian Society of Hematology guidelines for thalassemia and non-invasive iron measurements

A recent article by Angelucci *et al*.[1] offers recommendations for the diagnosis, monitoring, and management of thalassemia major and related disorders. The authors' recommendations were based on a literature survey that is itself a valuable resource for clinicians and researchers. By comparing non-invasive liver iron quantification by biomagnetic liver susceptometry (BLS) and magnetic resonance imaging (MRI), it was concluded that BLS using superconducting quantum interference devices (SQUID) was inaccurate and systematically underestimated liver iron concentration (LIC). Two studies were cited with extremely different correlations reported as coefficients of determination (R^2).[2,3] In one of the references, BLS had a very high correlation with wet-weight liver biopsy (R_2=0.98).[2] In the second reference, the correlation between LIC from dry-weight biopsy and by BLS is alleged to be extremely poor (R^2 = 0.21).[3] However, this value cannot be found in that citation nor can it be derived from any given data elsewhere.[4] Most likely, the regression coefficient of 0.46 (functional relationship between LIC from BLS and biopsy) was mistaken for a correlation coefficient R.

In fact, from the mentioned larger study group (blind substudy within the CICL670A0107 trial) R^2=0.74 for BLS and R^2=0.75 for MRI-R2 could be calculated (Pearson's correlation). Both methods rely on the large magnetic susceptibility of the hemosiderin/ferritin iron complex,[4] directly measured by BLS or via its interference with the transversal proton relaxation rate R2 or R2* by MRI. The authors should have wondered about such a correlation difference between these two methods. Consequently, a high correlation (R^2=0.86) was directly observed between BLS and MRI-R2 using the same MRI sequence as above.[5] Although we previously clarified the problem of under- or overestimation of LIC by BLS or from dry-weight liver biopsy,[6] the underestimation aspect appears again as a critical factor. In the CICL670A0107 substudy, a relationship of 0.46 was observed between LIC from *in vivo* BLS converted by a factor of 3.33 to a dry-weight equivalent LIC from deparaffinized biopsies.[3] Both BLS and MRI are non-invasive probes that determine the volume concentration of iron in living tissue. Thus, LIC from wet-weight biopsies would be the adequately corresponding parameter for calibration or validation.

Despite the similarities between MRI and BLS, there is a marked technical distinction between them, namely, the physics of the BLS signal is simple and well understood,[4] but the MRI signal is subject to a number of complicating physical factors, which make it impractical to calculate the iron concentration in an analytic way. As a result, MRI is forced to rely on biopsy data for calibration. In marked contrast, the BLS signal can be reliably calculated from magnetic flux integrals and the instrument can be calibrated without reference to biopsy data. This BLS calibration has been validated by comparing to LIC from wet-weight biopsies.[2,7] Problems with validation have certainly arisen in the past when non-invasive iron measurement methods like BLS were incautiously compared with LIC from dry-weight biopsies. On the other hand, liver iron is primarily measured in heat-dried biopsies either from fresh-tissue or paraffin-blocks. A critical investigation of the relationship between different liver biopsy preparations was recently performed resulting in a wet-to-dry weight ratio of 5.7±1.4 to 6.3±0.8 for deparaffinized, heat-dried liver samples depending on the drying temperature.[8] As the wet-weight of liver samples may be affected by different biopsy techniques and analysis in different laboratories, a blinded direct comparison between LIC by BLS and LIC from fresh-tissue heat-dried biopsies excised by cutting needle has been performed for the first time and a conversion factor of 6.1±0.3 (R^2=0.86) was obtained.[9] Without going into further details, this is in good agreement with the CICL670A0107 substudy.

We are running two of the 6-7 biosusceptometer facilities worldwide on a daily and routine basis, having used SQUID biosusceptometry in the diagnosis and follow-up of more than 3,000 patients, and were also involved in MRI-R2 measurements.[3,10] All iron measurement methods (liver biopsy, MRI and BLS) can be reliably used to monitor changes in patients with iron overload under treatment if applied with the necessary expertise. Especially in young patients and in repeated annual monitoring of iron stores, non-invasive methods have a clear advantage. Unfortunately, SQUID-BLS depends on dedicated equipment which is available only in a few specialized centers, although this may change in the future with the incorporation of high-temperature superconducting technology into BLS.[4] MRI technique would be available in many more locations worldwide, however, it still requires special and long-standing interest of radiologists, MRI imager time in competition with the hospital's schedule, and stable long-term machine performance, which together may turn out to be a restricting factor in practice. In summary, while Angelucci *et al.* provide a valuable general survey of the field, the recommendation with regard to non-invasive iron measurements has serious shortcomings. In addition, thorough analysis of available evidence and technical expertise of used methods are also needed before judging the accuracy of specific methods. Although serum ferritin reflects the relative change of body iron in response to iron chelator doses in large study groups,[11] it fails to reliably define absolute liver iron concentration or monitor changes of iron stores on an individual basis in patients with thalassemia.[12] Non-invasive techniques for iron quantification are clearly needed to optimally follow patients under iron chelation treatment. BLS or MRI in specialized centers might be the best way to achieve this goal.

Peter Nielsen,[1] Rainer Engelhardt,[1] Regine Grosse,[1] Gritta Janka,[1] Paul Harmatz,[2] and Roland Fischer[1,2]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Children's Hospital and Research Center, Oakland, USA

Key words: iron, SQUID, magnetic resonance imaging, liver iron concentration, biopsy.

Funding: PN, GJ, PH and RF have been supported in part by research funding from Novartis Pharma. RG and RF received honoraria as speakers from Novartis Pharma.

Correspondence: Roland Fischer, Department of Paediatric Haematology and Oncology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52 D-20246, Hamburg, Germany. Phone: international +49.40.428032389. Fax: international +49.40.428034797. E-mail: fischer@uke.uni-hamburg.de, rfischer@mail.cho.org

Citation: Nielsen P, Engelhardt R, Grosse R, Janka G, Harmatz P, Fischer R. Italian Society of Hematology Guidelines for thalassemia and non-invasive iron measurements. Haematologica 2009; 94:294-295. doi: 10.3324/haematol.13600

References

1. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica 2008;93:741-52.
2. Brittenham GM, Farrell DE, Harris JW, Feldman ES, Danish EH, Muir WA, et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med 1982;307:1671-5.
3. Piga A, Fischer R, Harmatz P, St. Pierre TG, Longo F, Fung E, et al. Comparison of LIC obtained from biopsy, BLS and R2-MRI in iron overloaded patients with β-thalassemia, treated with deferasirox (Exjade, ICL670). Blood 2005;106: 755a.
4. Fischer R, Farrell D. Liver iron susceptometry. In: Andrew M, Nowak H, editors. Magnetism in Medicine: a handbook, completely revised and extended edition. Berlin: Wiley-VCH; 2007. p. 529-49.
5. Carneiro AAO, Fernandes JP, De Araujo DB, Elias J, Martinelli ALC, Covas DT, et al. Liver iron concentration evaluated by two magnetic methods: magnetic resonance imaging and magnetic susceptometry. Magn Reson Med 2005;54:122-8.
6. Fischer R, Harmatz P, Nielsen P. Does liver biopsy overestimate liver iron concentration? Blood 2006:108:1775-6; author reply 1776.
7. Fischer R, Engelhardt R, Nielsen P, Gabbe EE, Heinrich HC, Schmiegel WH, et al. Liver iron quantification in the diagnosis and therapy control of iron overload patients. In: Hoke M, Erne SN, Okada YC, Romani GL, editors. Biomagnetism: clinical aspects. Amsterdam: Elsevier; 1992. p. 585-8.
8. Ropert-Bouchet M, Turlin B, Graham G, Rabault B, Le Treut A, Brissot P, et al. Drying methods affect the wet:dry weight ratio of liver tissue samples and impact liver iron content (LIC) measurements. In: First Congress of the International BioIron Society; 2005 May 25. Prague, Czech Republic.
9. Pakbaz Z, Fischer R, Vichinsky E, Fagaly RL, Paulson DN, Pratt K, et al. Liver iron measurement by SQUID biosusceptometry compared to liver biopsy: a more accurate definition of optimal iron range. Blood 2007;106:786a.
10. Engelhardt R, Langkowski JH, Fischer R, Nielsen P, Kooijman H, Heinrich HC, et al. MRI-sequences for liver iron quantification: studies in aqueous iron solutions, iron overloaded rats and patients with hereditary hemochromatosis. Magn Reson Imag 1994;12:999-1007.
11. Cappellini MD, Cohen A, Piga A, Bejaoui M, Perrotta S, Agaoglu L, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with β-thalassemia. Blood 2006;107:3455-62.
12. Pakbaz Z, Fischer R, Fung EB, Nielsen P, Harmatz P, Vichinsky E. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. Pediatr Blood Cancer 2007;49:329-32.

Italian Society of Hematology guidelines for thalassemia and non-invasive iron measurements: Author reply

We greatly appreciated the comments of Dr Nielsen and colleagues on the guidelines for the management of iron overload in thalassemia we produced on behalf of the Italian Society of Hematology.[1] Dr Nielsen and colleagues are concerned about our interpretation of data regarding the accuracy of biomagnetic liver susceptometry (BLS) as a non-invasive method for assessing liver iron concentration. By analyzing the existing evidence, we relied on the only two references dealing with a correlation between BLS and liver iron concentration by biopsy in patients with thalassemia. Our conclusion on the inaccuracy of BLS was mainly grounded on a paper published as an abstract by Piga *et al.*[2] in which the sentence "*on average, the LIC data obtained from BLS and biopsy were related by a factor of 0.46*" was interpreted as 0.46 being the correlation coefficient of the two measurements. Thus, from this factor, we derived a R^2 of 0.21. We also relied on the conclusion of the abstract that states "*overall, LIC from biopsy was generally larger than that obtained from BLS*".

Regarding the use of SQUID/BLS after the first study published by Gary Brittenham in 1982,[3] no other published study has confirmed the capability of SQUID to predict hepatic iron concentration with adequate methods. Any validation study of a new diagnostic quantitative procedure must compare the new methodology with a reference *gold* standard. Particularly a determination coefficient (R^2) with a prediction interval (95% CI) should be reported.

Above all in this specific case the 95% prediction interval would be reasonably narrow not extending over the identified threshold for iron concentration tissue damage and death risk.[4] In the setting of iron overload, the reference standard is the validated biochemical determination of hepatic iron concentration on adequate, non cirrhotic, liver biopsy specimens.[5] We are not aware of any such study with results similar to that reported by Dr Brittenham with a similar 95% confidence prediction interval. Studies comparing SQUID/BLS with other technologies are of minor relevance. Moreover the cited debate on dry weight-wet weight relationship developed after an industry sponsored trial,[2] which, although important for future development, raises concern for the thousands of determinations performed for clinical practice before 2006.

In conclusion, although SQUID/BLS is a highly scientific methodology, because of the limited availability, the limited literature in peer reviewed journals, the reported difficulties, and the availability of other non-invasive methods (MRI-R2) it appears rational to recommend its utilization only inside clinical trials.

Emanuele Angelucci,[1] Giovanni Barosi,[2] Monia Marchetti,[2] and Sante Tura[3]

[1]Hematology Department and BMT Unit, Cancer Center "Armando Businco", Cagliari; [2]Laboratory of Clinical Epidemiology, Fondazione IRCCS Policlinico S. Matteo, Pavia; [3]Istituto di Ematologia ed Oncologia Medica "Seragnoli", Università di Bologna, Italy.

Key words: clinical practice guidelines, systematic review, thalassemia major, SQUID, BLS.

Correspondence: Emanuele Angelucci, MD. Hematology Department and BMT Unit, Cancer Center "Armando Businco", via Edward Jenner, 09121 Cagliari, Italy. E-mail: emnang@tin.it

Citation: Angelucci E, Barosi G, Marchetti M,and Tura S. Italian Society of Hematology guidelines for thalassemia and non-invasive iron measurements: Author reply. Haematologica 2009; 94:295-296. doi: 10.3324/haematol.2008.001651.

References

1. Angelucci E, Barosi G, Camaschella C, Cappellini MD, Cazzola M, Galanello R, et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders.