When diagnostics meets translational research: detection of hemoglobin fractions in cellular lysates from in vitro erythroid cultures by Capillarys 2 Flex Piercing analyzer (Sebia)

ANNAMARIA APRILE, GABRIELLA PASSERINI, MARIA DOMENICA CAPPELLINI, SARAH MARKTEL, FABIO CICERI, GIULIANA FERRARI, and FERRUCCIO CERIOTTI

MILAN AND ROME, ITALY

Detection of hemoglobin (Hb) variants represents an important issue for diagnosis and adequate treatment of hemoglobinopathies. The Capillarys 2 Flex Piercing analyzer (Capillarys) by Sebia is routinely used in our clinical laboratories to detect Hb variants in peripheral blood (PB). This automated method separates Hb fractions by capillary electrophoresis, giving a spectrophotometric measure of their relative proportion. The scientific research in the field of hemoglobinopathies needs robust procedures to evaluate the efficacy of experimental therapies, as gene therapy. We investigated for the first time the feasibility to use Capillarys on cellular lysates from in vitro erythroid cultures. Because total Hb concentration in erythroid lysates is up to 20-fold lower than in hemolysates from PB, we analyzed diluted blood samples, thanks to the manual mode included in the Capillarys setting. We compared analytical precision, accuracy, sensitivity, and specificity of this procedure to the automatic method, routinely used in diagnostics. For instance, adult Hb intra- and interassay precision were estimated as coefficient of variation 0.2% and 0.3%, respectively. The manual mode is less robust for detection of fractions <3% and the lower level of sensitivity is 2 g/L of total Hb. Specificity of manual and automatic settings was equivalent. We confirmed the performance of the method by analyzing erythroid lysates from thalassemic patients' cultures. Our study demonstrated that the Capillarys 2 Flex Piercing manual method is comparable to the automatic one. The analysis is very robust at low Hb concentrations, as in erythroid cultures from patients affected by hemoglobinopathies, representing a useful tool also in translational research. (Translational Research 2016;169:31–39)

Abbreviations: BM = bone marrow; Capillarys = Capillarys 2 Flex Piercing analyzer (Sebia); CV = coefficient of variation; CE = capillary electrophoresis; CLSI = Clinical and Laboratory Standards Institute; Hb = hemoglobin; HLA = human leukocyte antigen; HPLC = high-pressure liquid chromatography; LV = lentiviral vector; O.D. = optical density; PB = peripheral blood; RBCs = red blood cells

From the San Raffaele-Telethon Institute for Gene Therapy (TIGET), Division of Regenerative Medicine, Stem Cells and Gene Therapy, IRCCS San Raffaele Scientific Institute, Milan, Italy; University of Rome "Tor Vergata", Rome, Italy; Service of Laboratory Medicine, San Raffaele Hospital, Milan, Italy; Università di Milano, Ca Granda Foundation IRCCS, Milan, Italy; Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy; Immunohematology and Transfusion Medicine Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy; Vita-Salute San Raffaele University, Milan, Italy.

Annamaria Aprile and Gabriella Passerini contributed equally to the work.

Submitted for publication June 30, 2015; revision submitted October 12, 2015; accepted for publication October 23, 2015.

Reprint requests: Ferruccio Ceriotti, Service of Laboratory Medicine, San Raffaele Hospital, Via Olgettina, 60, Milan 20132, Italy; e-mail: ceriotti.ferruccio@hsr.it.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2015.10.007

AT A GLANCE COMMENTARY

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Background

In the last few years, the scientific research in the field of hemoglobinopathies needs robust procedures to evaluate at a preclinical stage the efficacy of experimental approaches, as gene therapy. Detection techniques imported from diagnostics often do not fit to volumes and concentrations of preclinical samples.

Translational Significance

Our study highlights the robustness of Capillarys 2 Flex Piercing analyzer by Sebia to process preclinical samples at low hemoglobin concentrations, as erythroid cultures from patients affected by hemoglobinopathies. This instrument represents a useful tool not only in diagnostics, but also in translational research.

INTRODUCTION

In the past few years, identification of structural hemoglobin (Hb) variants and thalassemias has become increasingly important in clinical laboratories for the early detection and the adequate treatment of hemoglobinopathies.[1] The use of conventional gel electrophoresis was replaced by more accurate high-throughput procedures, as high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE).[2-4] Automated CE is a screening technique, complementary to HPLC for the routine detection and measurement of Hb.[5] CE patterns are simple and easy to quantify, and several studies showed an excellent correlation between CE and HPLC for the qualitative and quantitative Hb analysis.[6-9]

The Sebia Capillarys CE method was approved in 2007 by the US Food and Drug Administration (FDA), for the evaluation of hemoglobinopathies.[10-12]

The Sebia Capillarys 2 Flex Piercing analyzer (Capillarys) is the updated model of the instrument.[13,14] The Hemoglobin(E) program of Capillarys is employed for routine use in our clinical laboratories to separate Hb fractions by CE and to give a spectrophotometric measure of their relative proportion, expressed as percentage of total Hb, in peripheral blood (PB).

In parallel with the evolution of ever more accurate techniques for diagnostics, also the scientific research in the field of hemoglobinopathies developed new experimental approaches, such as gene transfer strategies, aimed to treat these disorders. In this context, the efficacy of new therapies needs to be assessed in preclinical studies by robust analytical procedures.

Among hemoglobinopathies, β-thalassemia represents the most widespread disorder and is characterized by significantly reduced ($\beta+$ trait) or absent ($\beta0$ trait) synthesis of Hb β chains. Patients affected by the most severe form of the disease suffer from a profound anemia that leads to death, unless treated with regular blood transfusions.[15] So far, allogeneic bone marrow (BM) transplantation represents the only definitive cure, although limited to patients with compatible donors.[16] Gene transfer of the corrected β-globin gene and transplantation of autologous $CD34^+$ hematopoietic stem and progenitor cells might give new opportunities also to patients lacking an human leukocyte antigen-identical donor. Results from the first clinical trial in thalassemia showed the therapeutic potential of the gene therapy approach.[17] In this field, we demonstrated the efficacy of gene transfer into repopulating hematopoietic stem and progenitor cells from thalassemic mice and thalassemic patients of the lentiviral vector (LV) GLOBE, encoding for the human β-globin gene.[18-20] At the preclinical stage, a crucial predictive parameter that needs to be evaluated is the Hb level, produced by the erythroblastic progeny of patients' cells, cultured in vitro.

To this purpose, we investigated for the first time the feasibility to use Capillarys to perform the separation and relative quantification of Hb in lysates of in vitro erythroid cultures derived from healthy donors' and thalassemic patients' $CD34^+$ cells before and after gene transfer by the GLOBE LV. The in vitro erythroid differentiation protocol reproduces steps of physiological human erythropoiesis, although with a limited output of terminally differentiated reticulocytes and red blood cells (RBCs). The total Hb concentration in erythroid cell lysates might be as less as 1–3 g/L, depending on the culture protocol and the proportion of mature cells at the end of the culture, whereas in hemolysates from PB, after the automatic predilution of the whole blood, it ranges between 10 and 28 g/L. This represents the main obstacle for the analysis of these samples by the Capillarys instrument.

Exploiting the manual mode included in the Capillarys setting, we analyzed PB hemolysates diluted at low level of Hb, and we compared analytical precision, accuracy, sensitivity, and specificity of this procedure to the FDA certified automatic method. Because we are interested in monitoring the formation of adult Hb (HbA) tetramers after the genetic transfer of β-globin gene, we focused mostly on HbA analysis. We extended some analyses to HbF and HbA2 fractions because they result upregulated in our culture conditions and also to make our procedure feasible for other gene therapy