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Impact of molecular status on clinical management in children with histiocytosis treated according to POL HISTIO project

Anna Raciborska[1], Zofia Małas[1*], Andrzej Tysarowski[2], Katarzyna Seliga[2], Katarzyna Machnik[3], Jadwiga Wecławek-Tompol[4], Katarzyna Derwich[5], Iwona Rurańska[6], Katarzyna Muszyńska-Rosłan[7], Elżbieta Michalak[8], Wanda Badowska[9], Grażyna Karolczyk[10], Radosław Chaber[11], Katarzyna Drabko[12], Carlos Rodriguez-Galindo[13]

[1]Department of Oncology and Surgical Oncology for Children and Youth, Institute of Mother and Child, Warsaw, Poland
[2]Department of Cancer Molecular and Genetic Diagnostics, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[3]Department of Pediatrics and Oncology, Pediatric and Oncological Children's Center, Chorzów, Poland
[4]Department and Clinic of Pediatric Oncology, Hematology and Bone Marrow Transplantation, Wroclaw Medical University, Wrocław, Poland
[5]Department of Pediatric Oncology, Hematology and Transplantology, University of Medical Sciences, Poznań, Poland
[6]Department of Pediatric Hematology and Oncology, Silesian Medical University, Zabrze, Poland
[7]Department of Pediatric Oncology and Hematology, Medical University of Bialystok, Ludwik Zamenhof Children's Clinical Hospital, Białystok, Poland
[8]Department of Pathology, Institute of Mother and Child, Warsaw, Poland
[9]Department of Pediatric Oncology and Hematology, Department of Clinical Pediatrics, University of Warmia & Mazury, Provincial Specialist Children's Hospital, Olsztyn, Poland
[10]Division of Pediatric Hematology and Oncology, Children's Hospital, Kielce, Poland
[11]Department of Pediatric Oncohematology, Rzeszów University, Rzeszów, Poland
[12]Department of Pediatric Hematology, Oncology and Transplantology, Medical University of Lublin, Lublin, Poland
[13]St Jude Children's Research Hospital, Memphis, Tennessee, USA

Introduction

Histiocytic disorders are rare diseases which affect 1–2 in 1,000,000 people. This frequency increases to 5–9 in 1,000,000 children under the age of 15 [1–3]. At the origin of these diseases is a clonal proliferation of cells that morphologically and immunophenotypically resemble macrophage line cells. Excessive activation of the RAS/-mitogen-activated protein kinases (MAPK) signaling pathway lies at the base of the pathomechanism [2, 4, 5]. The current classification divides histiocytosis into five groups: C, H, L, M and R.

The C group represents cutaneous non-Langerhans cell histiocytosis (non-LCH) with or without major systemic components. The H group includes hemophagocytic lymphohistiocytosis syndromes. To the L group belong Langerhans cell histiocytosis (LCH), intermediate cell histiocytosis (ICH), Erdheim-Chester disease (ECD), and mixed LCH/ECD syndromes. To the M group belong malignant histiocytosis (primary and secondary). The R group represents Rosai-Dorfman disease (sporadic: classic, extranodal, with neoplasia or immune disease and unclassified and familial) [1, 6].

LCH is the most frequent histiocytosis in children. The spectrum of clinical symptoms ranges from unifocal

*Address for correspondence: Zofia Małas, Department of Oncology and Surgical Oncology for Children and Youth, Institute of Mother and Child, Kasprzaka 17a, 01–211 Warsaw, Poland, phone +48 22 327 72 05, fax +48 22 632 98 51, e-mail: zofia.malas@imid.med.pl

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lesions, single organ or single system disease, to very serious life-threatening multisystemic multifocal diseases with the involvement of life-critical organs (highest risk organs: bone marrow, liver, and spleen). The course of these diseases varies from spontaneous remission to aggressive progression with life-threatening conditions including death [1, 2]. Therefore, the management of LCH can vary greatly from a 'wait-and-watch' attitude through serious procedures such as intensive chemotherapy up to bone marrow stem cell transplantation. In LCH, *BRAF* V600E is the most frequently detected mutation, followed by *MAP2K1* alterations [1, 2, 5, 7, 8]. The occurrence of mutations seems to cause a more severe course and resistance to conventional chemotherapy, with potentially higher mortality and severe secondary conditions such as neurodegenerative disease (ND) or sclerosing cholangitis (SC) with consequent liver cirrhosis [7, 9–12].

The mutations can be detected from tissue, but recently it has proved possible to also detect them from peripheral blood in some patients (circulating cell-free DNA — cfDNA extracted from the blood sample), which is called liquid biopsy. Among the methods used to detect circulating cfDNA are allele-specific real-time polymerase chain reaction (PCR) and digital droplet PCR (ddPCR).

This has brought about the possibility of monitoring the minimal residual disease (MRD) of histiocytic disorder, which means that it is possible to assess disease activity and the response to treatment at the molecular level, before the occurrence of clinical signs [13]. On the other hand, the presence of mutations allows for inclusion in chemotherapy-resistant cases treatment schedule targeted drugs very often.

It is known that mutations are detected in nearly 80% of histiocytic disorders, so in our trial we wanted to examine tfrequency of mutations in Polish children with histiocytic disorders in correlation with clinical conditions and the form the disease takes initially. Moreover, we aimed to assess the course of the disease in terms of detection of mutations in peripheral blood during observation or treatment.

Aim

In this study, we have summarized the impact of molecular status on clinical management in children with histiocytic disorders, treated according to the POL HISTIO project. This is the first non-commercial clinical trial aimed at optimizing the diagnosis and treatment of juvenile patients with histiocytic disorders in Poland.

Materials and methods

Between April 2021 and January 2023, 57 patients with histiocytic disorders were diagnosed and managed according to the POL HISTIO project (a non-commercial clinical trial financed by the Medical Research Agency; ABM). All patients included in the study required a histological confirmation of a histiocytic disorder (excluding hemophagocytic lymphohistiocytosis) at the time of diagnosis. Diagnosis of histiocytosis was performed using standard immunohistochemistry definitions. Physical examination and laboratory evaluation were performed during the staging or when indicated. Also, all patients had standard tumor imaging using X-ray, computed tomography (CT), magnetic resonance imaging (MRI), bone scan, or positron emission tomography (PET), as indicated, to assess the clinical status of the disease.

Furthermore, molecular studies were performed. Molecular status was assessed in tissue samples from all patients. This material was reviewed by a pathologist who marked the tumor area based on a hematoxylin and eosin (H&E) stained slide with not less than 20% tumor cells. Subsequently, three 10 µm slices from formalin-fixed paraffin-embedded (FFPE) tissue were obtained for the sample preparation. DNA was extracted from FFPE tissue using a Sherlock AX — A&A Biotechnology Kit and quantified using a Quantus Fluorometer. The tissue sample was initially tested using the quantitative polymerase chain reaction (qPCR) method and subsequently using the ddPCR method.

In addition, the presence of the mutation in circulating cfDNA from peripheral blood was assessed. Patient blood was monitored every three months or when indicated, provided that a mutation had been identified in the tissue. Peripheral blood (10 mL) was collected into PAXgene ccfDNA tubes supplied by Qiagen. Isolation was performed using the QIAamp Circulating Nucleic Acid Kit with a 5 mL volume of obtained plasma. Tests were performed using the ddPCR method (Figure 1). The results of molecular tests were analyzed as a predictor of the form and course of the disease, and their impact on therapeutic decision-making and treatment.

Results

The median age at the time of diagnosis was 3.5 years. 48 patients (84.2%) were diagnosed with LCH and nine with multifocal juvenile xanthogranuloma (JXG). 45 (78.9%) were classified as having a single-system disease (SS). High-risk organs were involved in four (7%) patients.

BRAF V600E mutation was detected in the tissue of 29 (51%) patients. 21 of them (72%) were aged 5 years or younger and the other eight (28%) were 6 or more. Nine of them (31%) were initially positive for cfDNA in peripheral blood. In another five cases (so together in 14 patients — 48.4%) *BRAF* V600E mutation in cfDNA was detected occasionally during observation and it correlated with clinical features of progression or resistance to conventional chemotherapy. Of the 29 initially *BRAF*-positive patients, 10 (34.5%) had the multi-system form, and 19 (65.5%) had the single-system form. Of the 28 initially *BRAF*-negative

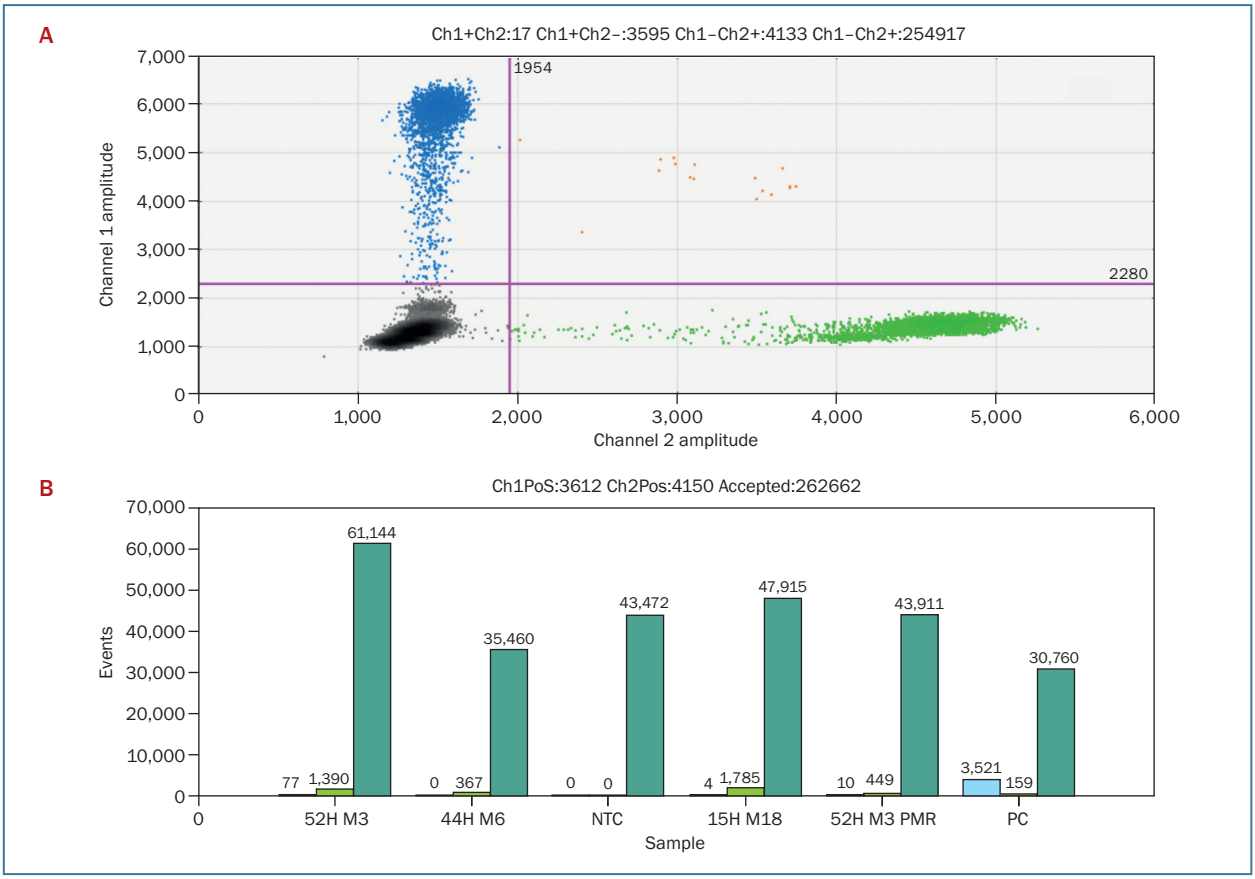

Figure 1A. Cases marked with green dots represent wild type of BRAF. Blue dots represent presence of BRAF V600E mutation in Langerhans cell histiocytosis (LCH), and orange dots represent droplets containing both wild-type and V600E mutant DNA. Black dots represent droplets that did not contain DNA; **B.** Quantitative distribution in a group of patients with LCH. I) Number of detected copies of V600E variant, II) number of copies of wild-type DNA, III) Total number of droplets generated in droplet digital polymerase chain reaction (ddPCR) reaction

patients, four (14%) had the multi-system disease and 24 (86%) had the single-system form. 31 (54.4%) patients received conventional chemotherapy (first line according to LCH-IV) using the standard inclusion criteria. There were *BRAF*-positive patients in both the treated and the observed groups, according to the form of the disease. Overall survival (OS) was 1.0. Event-free survival (EFS) was 0.27 in *BRAF*-positive patients and 0.68 in *BRAF*-negative patients ($p = 0.09$).

In all patients, the first-line conventional treatment was initiated based on inclusion criteria according to the LCH-IV protocol (clinical status, laboratory, and imaging results). Systemic chemotherapy was used in 30 patients. 15 of these (50%) recovered without relapse or progression, of whom eight (53.3%) patients recovered and were *BRAF*-positive, and seven (46.7%) recovered and were *BRAF*-negative.

A relapse (or more than one relapse) was observed in 18 (31.6%) patients during observation or treatment, adequately to the applied criteria of management. The vast majority, 15/18 (83.3%), were initially *BRAF*-positive. In all cases, the next lines of conventional therapy were included, but in eight of them targeted therapy was needed due to resistance to conventional treatment.

All the relapsed or progressed patients recovered clinically, and six of them continue targeted therapy. In three of them, positive *BRAF* in cfDNA is still detected. Six patients from the analyzed group (10%) developed neurodegenerative disease (LCH-ND) and five of those six (83%) were *BRAF*-positive. In the one (1.7%) patient from the recruited group, sclerosing cholangitis with liver cirrhosis occurred (*BRAF* mutation was identified in this case).

During the whole observation (including treated patients), the status of circulating cfDNA in the initially *BRAF*-positive patients was monitored. In six patients (66.6%), *BRAF*-positive cells monitored by cfDNA were eliminated. In three patients (33.3%), *BRAF* V600E in cfDNA is still present but without clinical manifestation of active histiocytosis. In 30 patients (53%), molecular status played a crucial role in the diagnostic process and the therapeutic decision-making, and had a significant impact on the choice of treatment.

Discussion

Histiocytic disorders are very rare neoplastic diseases with highly heterogeneous clinical manifestations. They can affect different organs and systems in uni-focal or multi-focal forms. The most common site involvements are the skin, bones, lymph nodes, liver, spleen, lungs, hematopoietic system, and central nervous system (CNS) [1, 7]. In 2010, Badalian-Very et al. [14] published a study describing the *BRAF* V600E mutation in CD1a+/CD207+ histiocytes. The *BRAF* gene is located on chromosome 7q34. It is part of the RAF kinase family [15]. Following that study, several trials were conducted focused on the mitogen-activated protein kinases (MAPK) pathway and alterations of the genes which are included in it. More alterations have been detected since 2010, and it is now known that some types of mutations could affect the clinical manifestation of histiocytosis. In the study by Kemps, clinicogenomic associations in childhood LCH were described. MAPK pathway gene alterations were detected in 79.6% of 377 patients with LCH, *BRAF* V600E in 50.7% of them, and other, even more rare, *BRAF* gene alteration in 14% [7].

We included a more heterogeneous group in our study, including patients with non-LCH, especially JXG, but the result is similar in patients with LCH (*BRAF* V600E detected in 58%). Today, the significance of detectable mutations in cfDNA is being examined. The prevalence of the alterations seems to be associated with the age distribution, the involved organs, the stage, and the response to first-line chemotherapy. Badalian-Very et al. [14] found the *BRAF* V600E mutations to be connected with the younger age of the patients, something also described by Hértier et al. [16].

In our study, we found that the overwhelming majority of patients with a *BRAF* V600E mutation were younger than 6 years. Wei et al. described a higher frequency of MS-LCH in *BRAF*-positive patients [17]. In our material, the patients were more diverse in terms of diagnoses, but the results were confirmed. Wang reported in his study that initially detectable cf*BRAF* V600E was more closely associated with important clinical characteristics, response to initial chemotherapy, and prognosis than lesion tissue *BRAF* V600E (lt*BRAF* V600E) alone [18]. Wang analyzed six patients, but in our study of 57 patients this observation was confirmed. In addition, the mutation status in peripheral blood seems to be a highly significant 'marker' of disease activity and treatment effectiveness in both conventional and targeted therapies.

Moreover, the fluctuation of *BRAF* mutation in cfDNA leads to a safer follow-up in untreated patients, with a relatively simple opportunity to collect reproducible material for monitoring.

Conclusions

Currently, the optimal diagnostic process includes molecular tests to decide on the best management and successful treatment. The best therapeutic decision can be made only by taking into account the clinical condition, the results of the pathological examination, and the laboratory and imaging tests with necessary molecular analysis. The most frequently identified mutation is *BRAF* V600E. The results of genetic alterations indicate patients at higher risk of a more severe course of the disease and at risk of secondary complications who require a change of standard treatment.

Moreover, monitoring the presence of the mutation in peripheral blood is an available method for early detection of disease recurrence or treatment resistance. Thus, the results of molecular tests may influence therapeutic decisions and treatment, including the possibility of targeted drugs.

Article information and declarations

Authors' contributions

AR, ZM, CRG — study concept and design. ZM, KM, JTW, KDe, IR, KRM, WB, GK, RC, KDr — shared patients' clinical data. EM — pathological analysis. KS, AT — molecular data and analysis. AR, ZM — interpretation of data and preparation of manuscript for publication. Final manuscript reviewed and approved by all authors.

Conflict of interest

The authors declare no conflict of interest.

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Approval and consent to participate

Written informed consent was obtained from all individuals included in the study and their parents or guardians on behalf of any participant under the age of 13. Approval for this retrospective study was obtained from all the relevant Institutions in compliance with international regulations for protection of human research subjects (Bioethics Committee no 44/2020).

Ethics statement

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments and uniform requirements for manuscripts submitted to biomedical journals.

Supplementary

There are no supplementary materials.

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