Human Pathology (2017) **60**, 95–103

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Spleen histology in children with sickle cell disease and hereditary spherocytosis: hints on the disease pathophysiology☆,☆☆

Marco Pizzi MD[a], Fabio Fuligni MS[b], Luisa Santoro MD[a], Elena Sabattini MD[c], Martina Ichino MD[d], Rita De Vito MD[e], Pietro Zucchetta MD[f], Raffaella Colombatti MD[g], Laura Sainati MD[g], Piergiorgio Gamba MD[d], Rita Alaggio MD[a,*]

[a]*Surgical Pathology and Cytopathology Unit, Department of Medicine—DIMED, University of Padova, 35121 Padova, Italy*
[b]*Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, M5G 1X8 Ontario, Canada*
[c]*Hematopathology Unit, Sant'Orsola/Malpighi Hospital, 40138 Bologna, Italy*
[d]*Pediatric Surgery Unit, Department of Woman and Child Health, University of Padova, 35128 Padova, Italy*
[e]*Department of Pathology, Bambino Gesù Children's Hospital, 00146 Rome, Italy*
[f]*Nuclear Medicine Service, Department of Medicine—DIMED, University of Padova, 35128 Padova, Italy*
[g]*Clinic of Pediatric Hematology/Oncology, Department of Woman and Child Health, University of Padova, 35128 Padova, Italy*

Received 4 May 2016; revised 17 September 2016; accepted 20 September 2016

Keywords:
Hereditary spherocytosis;
Sickle cell disease;
Spleen;
Vasculopathy;
Splenic pathophysiology

Summary Hereditary spherocytosis (HS) and sickle cell disease (SCD) are associated with splenomegaly and spleen dysfunction in pediatric patients. Scant data exist on possible correlations between spleen morphology and function in HS and SCD. This study aimed to assess the histologic and morphometric features of HS and SCD spleens, to get possible correlations with disease pathophysiology. In a large series of spleens from SCD, HS, and control patients, the following parameters were considered: (i) macroscopic features, (ii) lymphoid follicle (LF) density, (iii) presence of perifollicular marginal zones, (iv) presence of Gamna-Gandy bodies, (v) density of CD8-positive sinusoids, (vi) density of CD34-positive microvessels, (vii) presence/distribution of fibrosis and smooth muscle actin (SMA)–positive myoid cells, and (viii) density of CD68-positive macrophages. SCD and HS spleens had similar macroscopic features. SCD spleens had lower LF density and fewer marginal zones than did HS spleens and controls. SCD also showed lower CD8-positive sinusoid density, increased CD34-positive microvessel density and SMA-positive myoid cells, and higher prevalence of fibrosis and Gamna-Gandy bodies. HS had lower LF and CD8-positive sinusoid density than did controls. No significant differences were noted in red pulp macrophages. By multivariate analysis, most HS spleens clustered with controls, whereas SCD grouped separately. A multiparametric score could predict the degree of spleen changes irrespective of the underlying disease. In conclusion,

Abbreviations: HS, hereditary spherocytosis; LF, lymphoid follicle; MZ, marginal zone; RBC, red blood cell; SCD, sickle-cell disease; SMA, smooth muscle actin.

☆ Competing interest: No financial conflicts to declare.

☆☆ Funding/Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

* Corresponding author: General Pathology & Cytopathology Unit, Department of Medicine—DIMED, University of Padova, via Gabelli 61, Padova, PD 35121, Italy.

E-mail address: ral@unipd.it (R. Alaggio).

http://dx.doi.org/10.1016/j.humpath.2016.09.028

SCD spleens display greater histologic effacement than HS, and SCD-related changes suggest impaired function due to vascular damage. These observations may contribute to guide the clinical management of patients.

1. Introduction

The spleen is the largest organ of the lymphatic and reticuloendothelial system, and its functions include blood cell storage, hemocatheresis, and immune response against blood-borne infections [1]. These functions are sustained by 2 topographically and functionally distinct compartments: the white pulp and the red pulp [2].

The white pulp consists of periarterial sheaths of lymphocytes with evenly distributed LFs. Both primary and secondary LFs display an outer MZ, composed of small- to medium-sized lymphocytes with abundant cytoplasm. MZ lymphocytes exert a pivotal role in immune responses against polysaccharide-encapsuled bacteria (ie, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*) [3-5]. The red pulp consists of sinusoids surrounded by a meshwork of macrophages with interlacing cytoplasmic processes (red pulp cords). The histologic and ultrastructural features of the red pulp allow for the filtration of circulating blood cells and the removal of senescent and/or damaged erythrocytes [1,2].

Several hematologic disorders can alter the microscopic features of the spleen, with consequent damage to its immunologic and nonimmunologic functions. HS and SCD are rare congenital diseases, characterized by distinct pathogenic mechanisms and associated with variable spleen dysfunction [6,7]. HS is a genetic disorder of the erythrocyte cytoskeleton, causing abnormal red blood cell (RBC) shape, loss of RBC membrane, and chronic hemolysis [8]. The spleen is the main site of RBCs disruption, as spherocytes can hardly squeeze through the splenic sinusoids and are entrapped in the red pulp cords [1]. This causes moderate to severe splenomegaly, which is typically associated with anemia, reticulocytosis, jaundice, and increased risk of gallstones. Splenectomy improves HS-related symptoms in most cases [6,9].

SCD is a hereditary disorder caused by a point mutation on the *β-globin* gene, inducing a glutamic acid–to-valine substitution at position 6 [10]. The resulting hemoglobin (ie, hemoglobin S) is characterized by peculiar biochemical properties. It indeed displays a hydrophobic motif, which prompts the aggregation and precipitation of de-oxygenated hemoglobins in RBCs [11]. The precipitates promote the acquisition of a sickle-like shape, induce RBC hemolysis, and cause the entrapment of sickle cells in small vessels and capillary networks [12]. This leads to both intravascular and extravascular hemolysis, with vaso-occusive crises, microvascular thrombosis, and ischemic damage to several organs. SCD is indeed characterized by a broad spectrum of clinical manifestations, including pain crises, renal papillary necrosis, ischemic strokes, and bacterial infections. The latter are supposed to be mainly caused by the progressive shrinking of the spleen (ie, functional asplenia) possibly due to recurrent ischemic accidents of the red pulp [7]. In SCD, the spleen can also be involved by acute sequestration crises, which are characterized by a precipitous drop in hemoglobin concentration, reticulocytosis, and tender splenomegaly [13,14].

The morphologic and pathophysiologic bases of spleen dysfunction in HS and SCD are not fully described. Their better understanding may, however, contribute to improve the clinical management of patients. In the present study, the histologic features of HS and SCD spleens have been characterized by thorough morphometric and immunohistochemical analysis. The histologic results also allowed the development of a multiparametric score to assess the severity of splenic changes in each single case.

2. Materials and methods

2.1. Case selection

This retrospective, multi-institutional study considered a series of 42 spleens from pediatric patients (mean age, 8.6 years; male-to-female [M:F] ratio, 1.2) with HS and SCD, who underwent partial or total splenectomy for disease-related splenomegaly/hypersplenism. In detail, the following cases were considered: (i) 35 spleens from children with HS (total splenectomy: 32 cases; partial splenectomy: 3 cases), (ii) 7 spleens from children with SCD (total splenectomy in all cases; all spleens were removed as a consequence of previous sequestration crises), and (iii) 10 control spleens removed for traumatic rupture in pediatric patients (total splenectomy in all cases). The gross description (spleen weight, presence of subcapsular infarcts, thrombosis of hilar vessels) and clinicoepidemiologic data were available in all cases (Table 1).

Paraffin-embedded tissue blocks were retrieved from the archives of the Surgical Pathology & Cytopathology Unit of Padova University Hospital (Padova, Italy), the Hematopathology Unit of Bologna University Hospital (Bologna, Italy), and the Ospedale Pediatrico Bambino Gesù (Rome, Italy). All cases were reviewed by 2 pathologists (L. S. and M. P.), and representative tissue sections were selected for morphometric and immunohistochemical analyses. The ethics regulations on research on human tissues were followed by each of the participating centers, consistent with the Declaration of Helsinki.