The diagnostic role of Next Generation Sequencing in uncovering isolated splenomegaly: A case report

Giuseppe Auteri,1 Daniela Bartoletti,1 Clara Bertuzzi,2 Francesco Bacci,2 Valeria Tonini,3 Lucia Catani,1 Nicola Vianelli,1 Michele Cavo,1 Francesca Paladini1

1IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna; 2Hematopathology Unit, 3Emergency Surgery Unit, Sant’Orsola-Malpighi University Hospital, Bologna, Italy

Abstract

Many diseases can induce splenomegaly, however, about 5% of splenomegalies are idiopathic. When there is no underlying treatable cause, and the splenomegaly significantly affects the quality of life, splenectomy is the best therapeutic choice. A 67-year-old woman had idiopathic and asymptomatic splenomegaly. The increase in splenomegaly resulted in hypersplenism with cytopenia and symptoms related to abdominal discomfort. The patient underwent splenectomy which led to clinical improvement. A histological examination showed the presence of hematopoietic tissue. Peripheral blood Next Generation Sequencing with the myeloid panel SOPHIA Genetics showed the following mutations: ASXL1, SRSF2, KARS and TET2. Three out of these four mutations were also found in the splenic tissue. Next Generation Sequencing could be useful in the diagnosis of splenomegalies associated with myeloproliferative neoplasms otherwise defined as idiopathic, in order to address a therapeutic strategy.

Introduction

Splenomegaly is defined as spleen weight above 250 g,1 however various methods allow an indirect definition of splenomegaly (for example the longitudinal diameter over 11-14 cm, the spleen volume more than 314.5 cm³ calculated by ultrasound or CT).2,3 Splenomegaly is often a condition secondary to other diseases (Table 1).3 The differential diagnosis is often challenging and requires the involvement of numerous tools in terms of blood chemistry, histology and imaging.5,6

The spleen has a role in presenting antigens: an infection can stimulate a reactive splenomegaly, often reversible at the disruption of an antigenic stimulus. Consistently, even an autoimmune antigenic stimulus could result in reactive splenomegaly.

The spleen is in charge of the disposal of blood cells. Congenital blood cell disorders can cause splenomegaly, particularly during the first decades of life.

Congestion of portal venous flow or congestive heart failure may be responsible for splenomegaly, resulting in hypersplenism and consequent cytopenia. Hyperacumulation disorders are congenital or acquired and may induce organ failure or increase in volume. These diseases are usually associated with symptoms concerning other organs.

Neoplasms can induce splenomegaly due to metastasis or primitive splenic neoplasms, especially hematologic ones.7 Despite these causes, in about 5% of cases, splenomegaly remains idiopathic.5

In hematology, splenomegaly often underlies a neoplastic disease and its identification leads to a diagnosis and, therefore, to a therapeutic approach.

Potentially all lymphomas can be located in the spleen. Splenomegaly sometimes represents the first or the only sign of neoplasia. Except in rare cases (e.g. in splenic marginal zone lymphoma) splenectomy is not recommended and therapy of the underlying disease solves the splenomegaly.

Chronic Myeloid Leukemia (CML) is symptomatic in only 50% of cases, but splenomegaly is a frequent sign detected at diagnosis and the spleen size measured from the left costal margin is fundamental to calculate the Sokal score.8 The JAK1/2 inhibitor ruxolitinib was introduced into clinical practice, reducing spleen size and improving the quality of life in most patients.9

The diagnosis of MPNs were redefined by the WHO in 2016, giving a central role to the detection of JAK2, CALR and MPL mutations and to the histological examination of the bone marrow.10 About 2% of patient with Polycythemia Vera and 10% of patient with Essential Thrombocythemia and Myelofibrosis do not carry any of these mutations.

The so called “triple negative” patients show a worse prognosis in Leukemia Free Survival and the comprehension of the pathogenesis, diagnosis and management of these patients still represent an unmet clinical need.11

Next Generation Sequencing (NGS) allows the simultaneous analysis of a large quantity of samples and genes. The “Myeloid Solution” panel of SOPHIA genetics (Sophia Genetics, Saint Sulpice, Switzerland) analyzes 30 genes commonly involved in myeloproliferative neoplasms. Therefore, it is possible to have a lot of specific information about a patient in a relatively short time and at lower costs than techniques in which specific single genes are searched.

NGS permits to identify MPN patients with worse prognosis, especially “triple negative” ones and its use as a diagnostic tool is increasing.12

Case Report

We describe the case of a 67-year-old female that came to our observation because of a random detection of
In June 2017, the patient suffered from a severe dyspnea (NYHA class II/III) and asthenia. Complete blood count and laboratory evaluations were normal with the exception of a not clinically significant monoclonal IgG/k type peak (295 mg/dl), a rheumatoid factor and anti-nucleic autoantibodies. The patient had a multinodular thyroid goiter, she was obese and suffered from Sjogren disease requiring no therapy. The patient underwent a PET/CT which highlighted nodular overfixing to the medium lung lobe, bones and spleen. Bronchial cyto-morphology showed follicular bronchiitis with peri-bronchial metaplasia and foci of interstitial lymphoplasmacellular pneumonia. CT scan revealed a spleen longitudinal diameter of 25 cm, triggering a hematologic diagnostic work-up. On examination, grade 2 splenomegaly (2 cm from the costal arch) was noted. Thoracic and neurological examination were negative and there were no signs of heart failure.

### Table 1. Causes of splenomegaly.

| Causes of splenomegaly | Specific diseases |
|------------------------|------------------|
| Acute infection/infestation | Mononucleosis, Viral Hepatitis, Septicemia, Typhus, Toxoplasmosis |
| Subacute or chronic infection/infestation | Bacterial endocarditis, Brucellosis, Syphilis, HIV/AIDS, Plasmodium, Leishmania, Schistosoma |
| Autoimmune diseases | Rheumatoid Arthritis, Systemic Lupus Erythematosus, Rheumatic Polymyalgia, Systemic Sclerosis, Primitive Biliary Cirrhosis |
| Congenital blood cells disease | Hereditary Spherocytosis, Thalassemia, Sickled Cells disease |
| Systemic disease | Congestive Heart Failure, Portal Hypertension |
| Hyperaccumulation disorders | Sarcoidosis, Gaucher’s syndrome, Amyloidosis, Niemann-Pick syndrome, Wagner’s Granulomatosis |
| Cancer | Lymphomas, Chronic Myeloproliferative Neoplasms, Myelofibrosis, Polycythemia Vera, Essential Thrombocythemia, Chronic Myeloid Leukemia, Metastases, Primitive Splenic Neoplasms |
| Idiopathic |

Figure 1. Case report timeline Clinical/laboratory findings (left), diagnostic investigations (center), diagnostic/therapeutic approach (right), and timing (arrow). WBCs: White blood cells; Hb: hemoglobin; PLT: platelets; LCM: left costal margin; RBCs: red blood cells; PCR: polymerase chain reaction.
Infective and active autoimmune diseases were excluded.

Abdominal ultrasound showed an increased size and stiffness of the hepatic parenchyma (67kPa) with finely irregular profiles, thickened and granular echo-structure, arteriovenous hepatic fistula, hepatic lymph nodes with a reactive appearance, a splenic longitudinal diameter of 26 cm and signs of portal hypertension.

The BCR-ABL1 rearrangement and the mutations of JAK2V617F, CALR and MPL were absent.

In March 2018, mild leukocytosis (13.4×10⁹/L) with normal distribution of leucocytes subtype, anemia (10.1 g/dl) and thrombocytopenia (64×10⁹/L) occurred. No leukoerythroblastosis was detected and red blood cells shapes were normal.

A bone marrow biopsy was performed. The tissue was highly hyper-cellulated (98%), with a marked expansion of the myeloid precursors, with no blast cell. The cytogenetics could not be performed due to inconclusive marrow histology. A slight excess of CD34 + leukocytes in peripheral blood, detected with flow cytometry, was not suggestive for a diagnosis of Myelofibrosis with an MPN (0.11% of total peripheral cells).

Overall, the clinical and hematological picture was deemed to be secondary to cryptogenic cirrhosis. Splenomegaly further increased (from g III to g IV) and became symptomatic. Since the patient was transfusion-dependent and suffered from early satiety, splenic bulkiness, dyspnea and other symptoms related to anemia, she underwent splenectomy firstly with therapeutic purpose.

Splenectomy resulted in an improvement of anemia (10.4 g/dl), thrombocytopenia (173×10⁹/L) and symptoms.

The histological examination of the spleen, that had reached the maximum size of 35 cm, showed an extensive architectural effacement given by extramedullary hematopoietic tissue which replaced most of the regular splenic parenchyma (Figure 2).

At the time of splenectomy, a blood sample was collected for the search of myeloid mutations according to the “Myeloid Solution” panel of SOPHiA genomics. We observed a duplication in exon 12 of the ASXL1 gene with 30% of allele burden (p.Gly64Thr frameshift, c.1934dupG), a missense mutation in exon 1 of the SRSF2 gene with 50% of allele burden (p.Pro95Arg, c.284C>G), a missense mutation in exon 2 of the KRAS gene with 46% of allele burden (p.Gly12Arg, c.34G>C) and a missense mutation in exon 6 of the TET2 gene with 54% of allele burden (p.Cys1263Tyr, c.3788G>A).

Since hematopoietic tissue had been found in the spleen and the patient showed neutrophilic leukocytosis, as often happens in the MPN, we searched for the same mutations in the splenic tissue.

DNA was extracted from the formalin-fixed and paraffin-embedded samples and tested by control gene PCR (100-400 bp amplicons) in order to verify its integrity. Splenic DNA was analyzed with NGS. Three out of four of the mutations reported in peripheral blood were found (SRSF2, KRAS and TET2). The ASXL1 mutation probably was not found due to difficulties related to the DNA extraction method and to lower allelic burden.

The current patient’s clinical conditions are good. Splenomegaly-related symptoms are no longer present. The patient is anemic but no longer in need of transfusion. Hydroxyhurea is ongoing to control leukocytosis. The platelet count has normalized.

Discussion

Ruxolitinib is effective in reducing splenomegaly in most patients with MF and PV. However, in MF patients who are refractory to medical therapies, splenectomy may be useful to temporarily improve cytopenia and symptoms. In the present clinical case, the use of ruxolitinib was not possible due to the lack of a diagnosis of MPN and thrombocytopenia. A spleen biopsy would have exposed the patient to a high risk of bleeding. Because of the worsening of cytopenia and splenomegaly-related symptoms, splenectomy became the only executable treatment as well as diagnostic tool.

In this case, NGS was crucial for diagnostic definition of a splenomegaly associated with inconclusive marrow histology and molecular findings.

Figure 2. Splenic architectural effacement given by the heterotopic hematopoietic tissue with evident megakaryocytes (Hematoxylin and eosin staining). A) 20X magnification; B) 40X magnification.
Conclusions

NGS may represent a useful diagnostic and prognostic tool for patients with unexplained splenomegaly. Its implementation in internal medicine departments may be important to improve the management of patients with idiopathic splenomegaly.

In MPNs, the use of NGS helps to better stratify patients according to risk category and to choose more efficient therapeutic approaches such as hematopoietic stem cell transplantation.\(^\text{16-18}\)

In Myelofibrosis, the detection of \textit{ASXL1}, \textit{EZH2}, \textit{SRSF2} or \textit{IDH1/2} mutations define a high-risk patient.\(^\text{12}\) The ELN suggests transplantation in patients carrying high risk mutations, even when at intermediate-1 IPSS/DIPSS risk.\(^\text{18}\)

Evidence of a specific mutation would help identify specific drugs. For instance, a \textit{KRAS}-inhibitor that may be useful in our patient has been developed.\(^\text{19}\) Overall, the systematic research of myeloid panel mutations in NGS may improve the knowledge on MPNs pathogenesis and provide crucial information for clinical and therapeutical decisions.

References

1. Grover SA, Barkun AN, Sackett DL. The rational clinical examination. Does this patient have splenomegaly? JAMA 1993;270:2218-21.
2. Yetter EM, Acosta KB, Olson MC, Blundell K. Estimating splenic volume: sonographic measurements correlated with helical CT determination. AJR Am J Roentgenol 2003;181:1615-20.
3. Bezerra AS, D’Ippolito G, Faintuch S, et al. Determination of splenomegaly by CT: is there a place for a single measurement? AJR Am J Roentgenol 2005;184:1510-3.
4. Pozo AL, Godfrey EM, Bowles KM. Splenomegaly: investigation, diagnosis and management. Blood Rev 2009;23:105-11.
5. Sjoberg BP, Menias CO, Lubner MG, et al. Splenomegaly: A Combined Clinical and Radiologic Approach to the Differential Diagnosis. Gastroenterol Clin North Am 2018;47:643–66.
6. Motyczka G, Steensma DP. Why does my patient have lymphadenopathy or splenomegaly? Hematol Oncol Clin North Am 2012;26:395–408.
7. Bonnet S, Guédon A, Ribeirol JA, et al. Indications and outcome of splenectomy in hematologic disease. J Visc Surg 2017;154:421–9.
8. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in «good-risk» chronic granulocytic leukemia. Blood 1984;63:789–99.
9. Mesa RA, Gotlib J, Gupta V, et al. Effect of ruxolitinib therapy on myelofibrosis-related symptoms and other patient-reported outcomes in COMFORT-I: a randomized, double-blind, placebo-controlled trial. J Clin Oncol Off J Am Soc Clin Oncol 2013;31:1285–92.
10. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391–405.
11. Tefferi A, Guglielmelli P, Larson DR, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocytopenia, polycythemia vera, and myelofibrosis. Blood 2014;124:2507–13.
12. Guglielmelli P, Lasho TL, Rotunno G, et al. MIPPS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis. J Clin Oncol Off J Am Soc Clin Oncol 2018;36:310–8.
13. Passamonti F, Giesshammer M, Palandri F, et al. Ruxolitinib in the treatment of inadequately controlled polycythemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. Lancet Oncol 2017;18:88–99.
14. Mesa RA, Tefferi A. Palliative splenectomy in myelofibrosis with myeloid metaplasia. Leuk Lymphoma 2001;42:901–11.
15. Pottakkat B, Kashyap R, Kumar A, et al. Redefining the role of splenectomy in patients with idiopathic splenomegaly. ANZ J Surg 2006;76:679–82.
16. Bacher U, Shumilov E, Flach J, et al. Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use. Blood Cancer J 2018;8:113.
17. Alduaij W, McNamara CI, Schub A, et al. Clinical Utility of Next-generation Sequencing in the Management of Myeloproliferative Neoplasms: A Single-Center Experience. HemaSphere 2018;2:e44.
18. Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. Leukemia 2018;32:1057–69.
19. Ostrem JML, Shokat KM. Direct small-molecule inhibitors of KRAS: from structural insights to mechanism-based design. Nat Rev Drug Discov 2016;15:771–85.