Spontaneously Ruptured Spleen Samples in Patients With Infectious Mononucleosis

Analysis of Histology and Lymphoid Subpopulations

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ABSTRACT

Objectives: *Spontaneous rupture of the spleen is occasionally seen as the presenting event in infectious mononucleosis (IM). Diagnosis of these cases can be very challenging.*

Methods: *We describe the morphologic and immunohistochemical findings in a series of seven splenectomy specimens removed after spontaneous rupture in patients with IM. Most cases were submitted for a second opinion since the histology of the cases suggested malignant lymphoma.*

Results: *All the cases showed similar findings, with red pulp expansion occupied by activated T and B cells, including scattered large lymphocytes with both T- and B-cell markers, together with a polymorphic background rich in cytotoxic T cells. Clonality analysis revealed T-cell receptor clonal patterns in four of the six cases evaluated.*

Conclusions: *IM should be considered a possible diagnosis in any case of splenic rupture whose histology suggests possible aggressive lymphoma.*

Spontaneous rupture of the spleen has occasionally been reported as the presenting event in infectious mononucleosis (IM).[1,2] Up to 15% of splenic ruptures without risk factors or previously diagnosed disease have been attributed to IM.[2-4] Diagnosis of these cases can be challenging, since some of the histologic features of splenic involvement by IM may strongly suggest a malignant lymphoproliferative process.[5,6] The histologic features of IM tissue involvement have most often been described in lymph nodes and the Waldeyer ring[5] but only in isolated cases in the spleen.[7] We report morphologic and immunohistochemical findings in a series of seven cases in which the spleen was removed after spontaneous rupture in patients with IM; most cases were referred for a second opinion because of histologic findings that suggested a malignant lymphoproliferative process.

Materials and Methods

Paraffin-embedded blocks from seven formalin-fixed, paraffin-embedded cases were stained for H&E and for a panel of antibodies (listed in Table 1).

Clonality was analyzed by polymerase chain reaction (PCR) to detect immunoglobulin H (IgH) and TCR gene rearrangements by standard methods.[8] It was assayed by conventional methods using DNA extracted from formalin-fixed, paraffin-embedded tissue. Multiplex PCR to detect clonal VH-JH rearrangements was performed

Table 1
Antibodies and Probes Used in the Study

Antigen	Clone	Source
CD20	FLEX CD20cy, MxH L26	Dako Autostainer Plus (Dako, Carpinteria, CA)
PAX5	FLEX BSAP, MxH DAK-PAX5	Dako Autostainer Plus (Dako)
CD3	FLEX CD3, RxH	Dako Autostainer Plus (Dako)
CD2	FLEX CD2, MxH AB75	Dako Autostainer Plus (Dako)
CD4	FLEX CD4, MxH 4B12	Dako Autostainer Plus (Dako)
CD5	FLEX CD5, RxH 4C7	Dako Autostainer Plus (Dako)
CD8	FLEX CD8, MxH C8/144B	Dako Autostainer Plus (Dako)
Granzyme B	GRB1	Dako Autostainer Plus (Dako)
Perforin	5B10	Thermo Scientific, Waltham, MA (Ref: MS-1834-S1)
TIA1	TIA-1	Abcam, Cambridge, UK (Ref: ab2712)
CD56	FLEX CD56, MxH 123C3	Dako Autostainer Plus (Dako)
CD30	FLEX CD30, MxH BerH2	Dako Autostainer Plus (Dako)
κ	FLEX κ, RxH	Dako Autostainer Plus (Dako)
λ	FLEX λ, RxH	Dako Autostainer Plus (Dako)
PD1	PD1 clone: NAT105C/E3	CNIO, Madrid, Spain
Cyclin D1	FLEX cyclin D1, RxH SP4	Dako Autostainer Plus (Dako)
TCRGAMMA		Thermo Scientific (Ref: TCR1153)
TCRBF1	8A3	Thermo Scientific (Ref: TCR1151)
EBV-LMP1	CS1.4	Dako Autostainer Plus (Dako)
EBER	EBER CISH DAKO REF: Y5200	Dako Autostainer Plus (Dako)
Ki67	FLEX Ki67 antigen, MxH MIB-1	Dako Autostainer Plus (Dako)

using standardized Biomed2 primers, as fully described elsewhere.[9] We analyzed complete rearrangements of IgH (VH FR1-JH, VH FR2-JH, VH FR3-JH) and rearrangements of TCR (TCRG tube A [Vg-Jg], TCRG tube B [Vg-Jg], TCRB tube A [Vb-Jb], TCRB tube B [Vb-Jb], TCRB tube C [Db-Jb]). Results were interpreted following the recommendations of the Euroclonality/Biomed2 group.[10]

Results

Clinical Data

Patient age ranged from 15 to 25 years. Two of the patients described previous symptoms suggesting IM. Two of the patients died because of intra-abdominal hemorrhage and hypovolemic shock. Four patients, for whom follow-up data were available, remained alive and well after 3 to 240 months follow-up **Table 2**.

Spleen weight ranged from 276 to 460 g. A diagnosis of IM was established after clinical and serologic study, before or after splenectomy.

Description of the Histology

Splenic infarcts were present in all cases, together with subcapsular and intraparenchymal lacerations. Viable areas of the spleen had a homogeneous macroscopic appearance, without nodules. The number of studied blocks varied between hospitals; generally, we received one paraffin block from the spleen and additional blocks from any macroscopic change and hilar lymph nodes, when excised. Histologic study disclosed red pulp expansion with partial loss of the white pulp, a major finding in all cases. Focal geographic necrosis was seen in all cases. Trabecular and subendothelial polymorphic infiltration was present to a variable extent in all cases. Study of the red pulp disclosed sinusoidal and Billroth cord infiltration by polymorphic large lymphocytes, including scattered large mononuclear cells with a prominent nucleolus. Activated lymphocytes exhibited a wide range of cell sizes and shapes but did not form aggregates. In all cases, large activated lymphocytes were admixed with small lymphocytes, plasma cells, and numerous macrophages. Characteristic Reed-Sternberg binucleate cells were not seen, but mononuclear and multinucleate pleomorphic cells were found in every case. Scattered macrophages with cytoplasmic nuclear fragments were seen throughout the red pulp, in some cases with phagocyted RBCs. Prominent germinal centers were not seen in any case, although reactive follicles contained some residual germinal centers **Image 1**.

Immunohistochemical study revealed a majority of cytotoxic T lymphocytes, positive for CD3, CD2, CD5, CD8, and cytotoxic granules (TIA1, granzyme B, perforin) **Image 2**, **Image 3**, and **Table 3**. Scattered CD30-positive large lymphocytes were found in the red pulp. Large transformed cells showed either B-cell (CD20) or T-cell (CD3) markers. Immunoblasts and large activated lymphocytes were mostly positive for MUM1, were negative for BCL-6 and CD10, and showed weak BCL2 staining. Epstein-Barr virus (EBV)-encoded small RNA (EBER)-positive cells were seen mainly in the red pulp, homogeneously distributed. EBV/latent membrane

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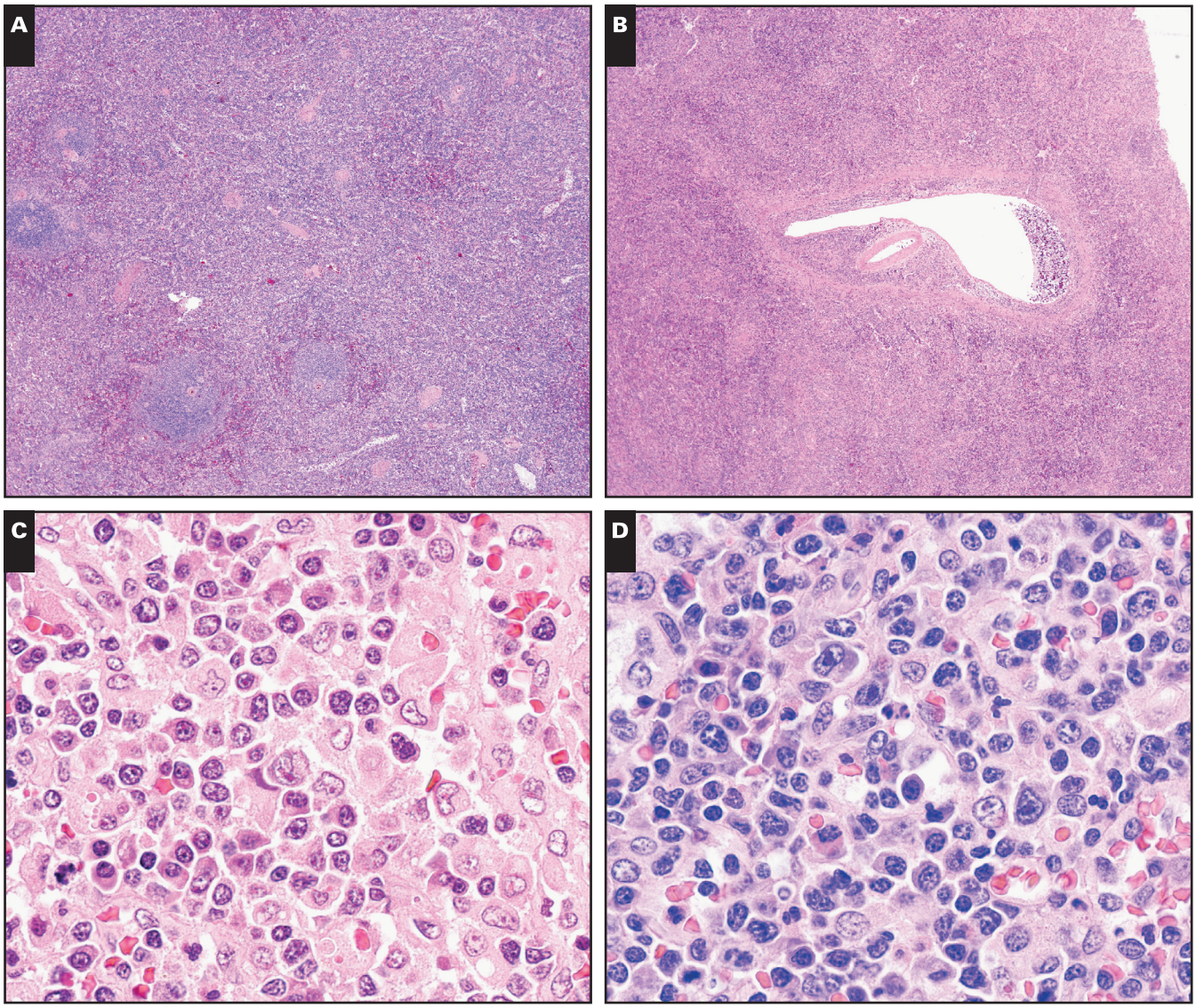

Image 1 Red pulp expansion (**A**), with striking subendothelial infiltration (**B**), by a polymorphic background rich in large pleomorphic cells, macrophages, plasma cells, and small lymphocytes (**C-D**).

protein 1 (LMP1) immunostaining showed isolated rare positive cells in all cases. Staining for κ and λ showed cytoplasmic staining by some of the activated B cells, without light chain restriction.

Active caspase 3 staining revealed strong staining in the nuclei of endothelial cells in scattered vessels (Image 3).

Differential diagnosis with Hodgkin lymphoma and diffuse large B-cell lymphoma was also considered necessary. Polymorphic histology and the presence of Sternbergoid cells forced us to consider the possibility of Hodgkin lymphoma, but the lack of a small lymphocyte background and the absence of a classic Hodgkin lymphoma immunophenotype (CD30+, CD15+, PAX5+, EBV/LMP) argued against this diagnosis. A possible diagnosis of diffuse large B-cell lymphoma was also considered in all cases, but polymorphic histology, the presence of activated T cells, and scattered apoptosis were strongly considered evidence against such a possibility.

Molecular Studies

Clonality analysis revealed TCR clonal patterns in four of the six cases evaluated. Clonal T-cell populations were detected in every positive case in a single gene locus (isolated TCR rearrangements) Figure 1. Three of six cases showed complete TCR G/B gene rearrangements (two G-B and one B-B). One case showed incomplete TCR rearrangements (B-C). The pattern of TCR rearrangements was polyclonal or nonclonal in the other two cases. IgH clonality analysis identified polyclonal B-cell populations in all six available cases.

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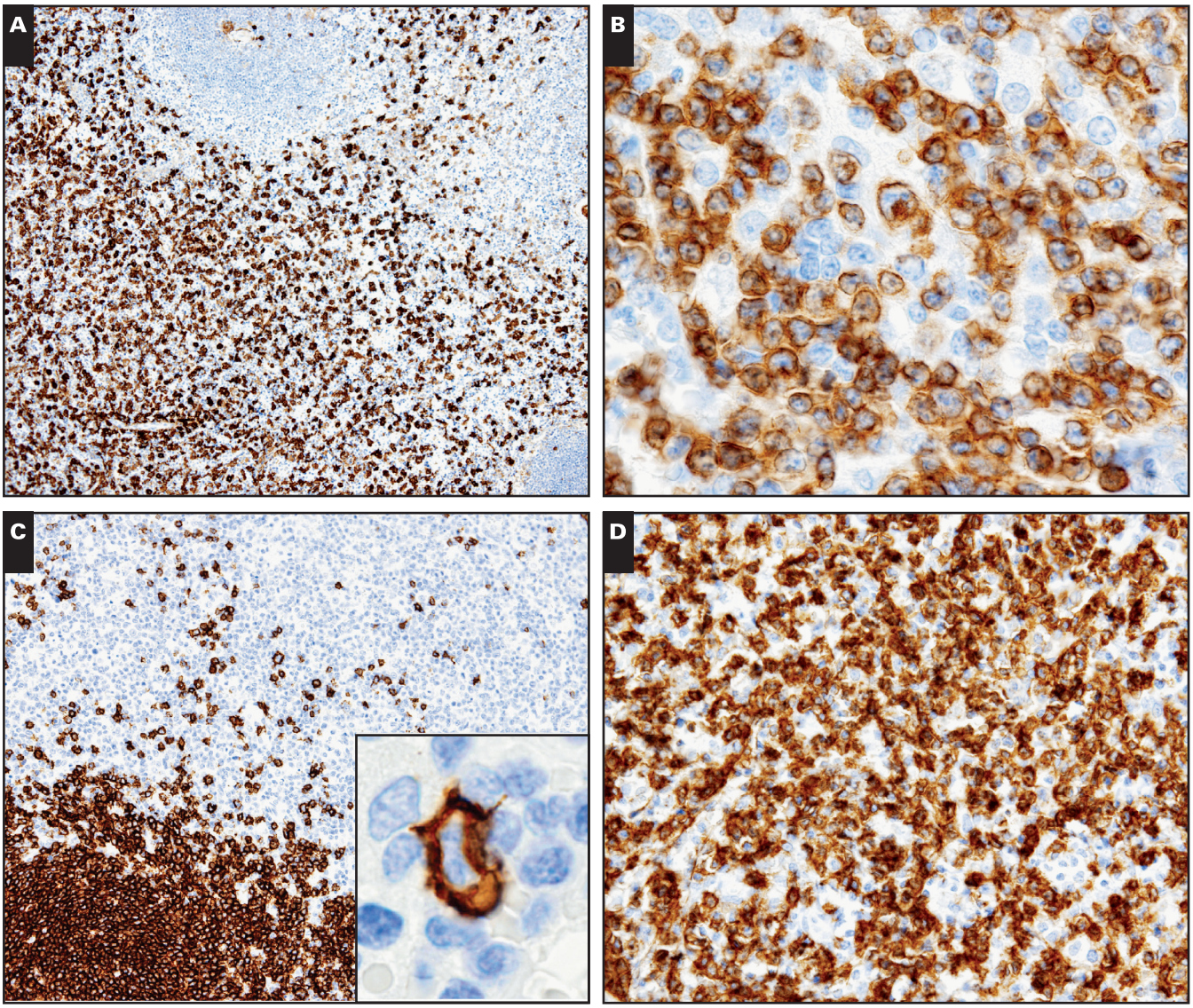

Image 2 Immunohistochemical study disclosed numerous CD163-positive macrophages (**A**), a rich CD3-positive T-cell infiltrate (**B**), with a striking presence of scattered large B cells (case 2) (**C**) and CD8-positive T cells (**D**).

Discussion

Spontaneous rupture of the spleen is a well-established complication of IM. Nevertheless, descriptions of spleen histology are scarce, and few individual cases have been described, to our knowledge.

Although some of the patients with this complication may have been previously diagnosed with IM, cases may arrive at a pathology department without appropriate clinical information and receive an incorrect diagnosis. Indeed, most of the cases described here were initially considered possible lymphoma cases, and their tissue samples were sent abroad for a second opinion.

During the same period, we have had the opportunity to review approximately 700 cases of spleen specimens involving different lymphoproliferative processes, derived from our own files and consultation cases from many Spanish hospitals. Therefore, spleen specimens in patients with IM represent roughly 1% of the examined splenectomy specimens.

As Table 2 shows, most of the cases are from pediatric patients (four of six), so we might expect the frequency of splenectomy specimens involving IM to be higher in pediatric hospitals or pathology departments receiving mainly specimens from pediatric patients.

All the cases considered here yielded similar findings, with red pulp expansion, which appears to be occupied by activated T and B cells, together with a polymorphic background including macrophages, small lymphocytes, and plasma cells. EBV-positive cells were a minor component in all cases, comprising

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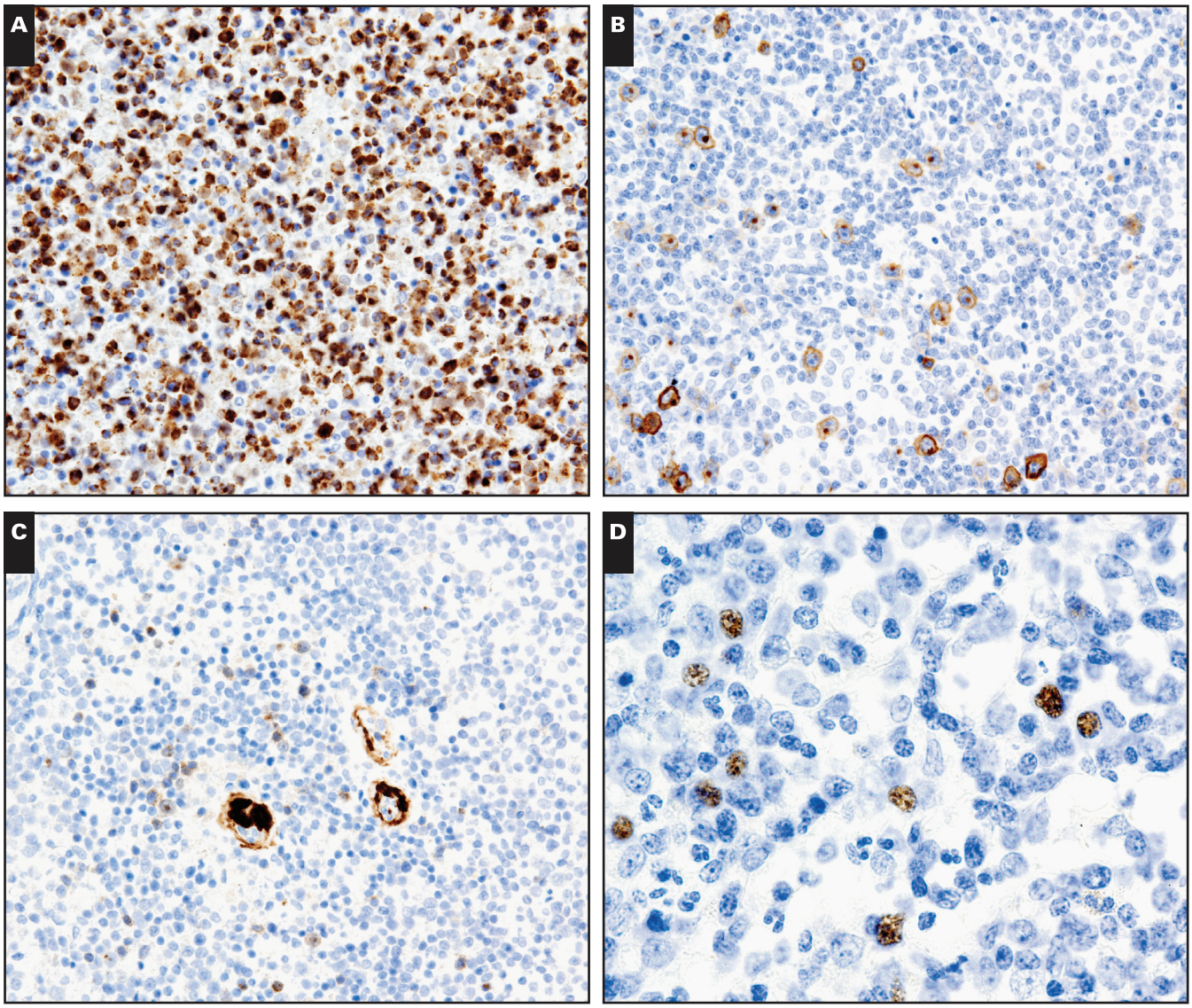

Image 3 Some of the most striking findings emerge from the immunohistochemical study. **A**, T-cell infiltrate is mostly composed of cytotoxic T cells (x200). **B**, Scattered large lymphocytes positive for CD30 (x200). **C**, Endothelial cells may show intense active caspase 3 staining (x200). **D**, EBER staining present in scattered cells (case 3) (x400).

Table 2
Relevant Clinical Data in the Series of Cases[a]

	Case No.						
Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Age, y	NA	17	24	15	25	16	17
Sex	M	F	M	F	M	NA	F
Spleen size	NA	18 × 18 × 3 cm	NA	14 × 11 × 3.5 cm	460 g	20 × 10 × 4.5 cm, 330 g	18 × 10 × 4 cm, 350 g
Follow-up	NA	A&W, 60 mo	A&W, 91 mo	A&W, 240 mo	A&W, 3 mo	DOD, 0 mo	DOD, 0 mo

A&W, alive and well; DOD, died of disease; NA, not available.
[a] Clinical data for case 1 are missing.

fewer than 20% of cells in the spleen; most of the infiltrate was therefore made up of a complex inflammatory background rich in cytotoxic T cells and macrophages. Significantly, both B- and T-cell activated large cells were identified, a finding that, in our experience, often supports the suspicion of IM.

The mechanism of splenic rupture in IM is unclear but may be related to the sudden development of

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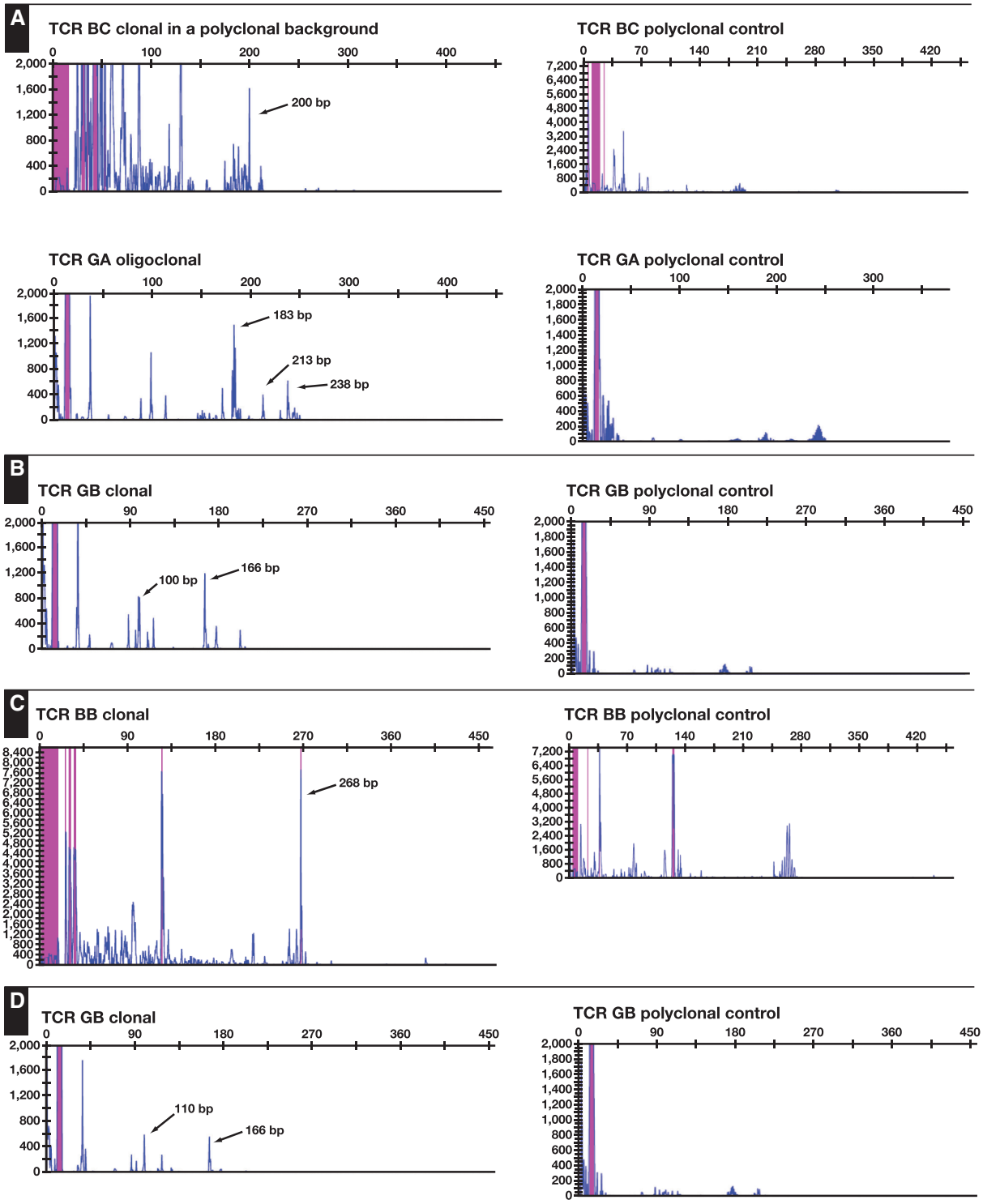

Figure 1 **A**, Case 3. GeneScan plots of T-cell receptor (TCR) BC and TCR GA tubes are shown. A single peak (200 base pairs [bp]) in a polyclonal background is found in TCRB tube C (Db-Jb) and a oligoclonal pattern is found in TCRG tube A (Vg-Jg). The other tubes (TCR-GB, TCR-BA, TCR-BB) showed a polyclonal pattern. **B**, Case 4. GeneScan plots of TCR GB (Vg-Jg) tube are shown. A clonal pattern with two dominant peaks is found (100, 166 bp). The other tubes (TCR-GA, TCR-BA, TCR-BB, TCR-BC) showed a polyclonal pattern (not shown). **C**, Case 5. GeneScan plots of TCR BB (Vg-Jg) are shown. A single clonal peak (268 bp) is found. The other tubes (TCR-GA, TCR-GB, TCR-BA, TCR-BC) showed a polyclonal pattern (not shown). **D**, Case 6. GeneScan plots of TCR GB (Vg-Jg) are shown. A clonal pattern (two peaks; 110, 166 bp) is found. The other tubes (TCR-GA, TCR-BA, TCR-BB, TCR-BC) showed a polyclonal pattern (not shown).

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Table 3
Histologic and Immunohistochemical Data

	Case No.						
Feature	**1**	**2**	**3**	**4**	**5**	**6**	**7**
B cells, %	5	10	10	10	10	10	10
T cells, %	60	60	60	60	60	60	60
CD4, %	10	10	10	10	10	10	5
CD8, %	50	50	50	50	50	50	50
CD30, %	NA	20	20	20	20	20	20
EBER+ cells, %	20	5	5	5	5	5	5
EBV/LMP+ cells, %	NA	2	<1	<1	<1	<1	<1
Cytotoxic markers, %	NA	50	40	40	50	40	40
CD56, %	NA	<1	<5	5	5	<5	5
TCR, γ/δ	NA	2	2	5	5	2	2
TCRBF1, %	NA	NV	NV	NV	60	NV	50
PD1, %	NA	30	50	40	40	30	40
Macrophages, %	NA	20	20	20	20	20	20
Plasma cells/CD138, %	NA	20	25	20	20	20	20
Light chain restriction, κ/λ	NA	Polytypic	Polytypic	Polytypic	Polytypic	Polytypic	Polytypic
TCR PCR	NA	Polyclonal	Isolated clonal peak	Isolated clonal peak	Isolated clonal peak	Isolated clonal peak	Nonclonal
β, A		Nonclonal	Nonclonal	NV	Nonclonal	NV	Nonclonal
β, B		Polyclonal	Nonclonal	Nonclonal	Clonal	Nonclonal	Nonclonal
β, C		Polyclonal	Clonal	Polyclonal	NV	Polyclonal	Nonclonal
γ, A and B		NV	NV	γA: polyclonal γB: clonal	NV	γB: clonal	γA: nonclonal γB: nonclonal
IgH PCR (FR1, FR2, FR3)	NA	Nonclonal/ polyclonal	Nonclonal/ polyclonal	Nonclonal/ polyclonal	Nonclonal/ polyclonal	Nonclonal/ polyclonal	Nonclonal/ polyclonal
Ki67, %	NA	50	50	50	55	30	30

EBER, Epstein-Barr virus-encoded small RNA; EBV, Epstein-Barr virus; IgH, immunoglobulin H; LMP, latent membrane protein; NA, not available; NV, not valid; PCR, polymerase chain reaction; TCR, T-cell receptor.

Table 4
Useful Data for Infectious Mononucleosis Recognition in Splenectomy Specimens

Young patients with sudden splenic rupture
Relatively small splenectomy specimens with infarcts and subcapsular and intraparenchymal lacerations
Polymorphic histology with striking apoptosis
Mixture of large activated B and T cells, scattered CD30-positive cells
Lack of classic morphologic or immunohistochemical features of Hodgkin lymphoma or large B-cell lymphoma

splenomegaly, since the spleen is much smaller than in other low-grade lymphomas involving the spleen. Destruction of support elements, such as vessels or the connective trabeculae, could also play a role.

Spontaneous rupture of the spleen can also occur in large B-cell lymphoma splenic infiltration, a diagnosis that we excluded because of the polymorphic background, EBV presence, and lack of immunoglobulin gene monoclonal rearrangements. Differential was also performed with splenic involvement by angioimmunoblastic T-cell lymphoma, in which EBV-positive scattered B cells can also be observed, although we are not aware of cases of splenic spontaneous rupture in this condition.

IM should be considered a possible diagnosis in any case of splenic rupture whose histology may suggest aggressive lymphoma **Table 4**.

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References

1. Bartlett A, Williams R, Hilton M. Splenic rupture in infectious mononucleosis: a systematic review of published case reports. *Injury.* 2016;47:531-538.
2. Aubrey-Bassler FK, Sowers N. 613 Cases of splenic rupture without risk factors or previously diagnosed disease: a systematic review. *BMC Emerg Med.* 2012;12:11.
3. Rutkow IM. Rupture of the spleen in infectious mononucleosis: a critical review. *Arch Surg.* 1978;113:718-720.
4. Smith EB, Custer RP. Rupture of the spleen in infectious mononucleosis: a clinicopathologic report of 7 cases. *Blood.* 1946;1:317-333.
5. Louissaint A Jr, Ferry JA, Soupir CP, et al. Infectious mononucleosis mimicking lymphoma: distinguishing morphological and immunophenotypic features. *Mod Pathol.* 2012;25:1149-1159.
6. O'Malley DP GT, Orazi A. *Atlas of Nontumor Pathology, First Series, Fascicle 7, Benign and Reactive Conditions of Lymph Node and Spleen*. Washington, DC: Armed Forces Institute of Pathology; 2009:283-294.
7. Heo DH, Baek DY, Oh SM, et al. Splenic infarction associated with acute infectious mononucleosis due to Epstein-Barr virus infection. *J Med Virol.* 2017;89:332-336.
8. Evans PA, Pott Ch, Groenen PJ, et al. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets: report of the BIOMED-2 concerted action BHM4-CT98-3936. *Leukemia.* 2007;21:207-214.
9. van Krieken JH, Langerak AW, Macintyre EA, et al. Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 concerted action BHM4-CT98-3936. *Leukemia.* 2007;21:201-206.
10. Langerak AW, Groenen PJ, Brüggemann M, et al. Euroclonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia.* 2012;26:2159-2171.

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