1-1-2014

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Diagnostic Challenges and Pitfalls

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• Hepatosplenic T-cell lymphoma is a rare and aggressive peripheral T-cell malignancy that is distinctively characterized by sinusoidal infiltration of mature medium-sized T lymphocytes in the spleen and liver. The neoplastic cells are classically surface $CD3^+$, $CD2^+$, $CD5^-$, $CD4^-$, and $CD8^{+/-}$ and manifest variable expression of markers associated with natural killer (NK) cells such as CD16 and CD56. In this article, we report the first case to date of a newly diagnosed de novo surface $CD3^-$ hepatosplenic T-cell lymphoma with circulating blastlike neoplastic cells expressing NK-cell–associated markers. The lack of surface CD3 expression, together with the expression of NK-cell–associated markers and the leukemic presentation, leads to significant diagnostic challenges in differentiating this $CD3^-$ hepatosplenic T-cell lymphoma from NK-cell neoplasms, in particular aggressive NK-cell leukemia. The related literature is reviewed, and the approaches for adequate diagnosis of this novel situation are described.

(*Arch Pathol Lab Med.* 2014;138:969–973; doi: 10.5858/arpa.2013-0074-CR)

Since the report of the first case in 1990 by Falco et al, approximately 150 cases of hepatosplenic T-cell lymphoma (HSTCL) have been reported, accounting for less than 5% of all peripheral T/NK (natural killer)–cell lymphomas.[1,2] Hepatosplenic T-cell lymphoma occurs predominantly in young men at a median age of 35 years.[3] Twenty percent of the cases arise in patients with a history of immunosuppression such as (but not limited to) organ transplantation, Crohn disease, hepatitis B infection, and intake of immunosuppressive drugs such as azathioprine and additional medications used to treat rheumatoid arthritis and systemic lupus erythematous.[1–4] Unlike other immune-mediated lymphomas, Epstein-Barr virus (EBV) infection is not associated with the disease.[3] Most of the cases are positive for $\gamma\delta$ T-cell receptor (TCR) gene rearrangement and expression, although a few $\alpha\beta$ TCR expressions have been reported recently.[1] Patients with hepatosplenic T-cell lymphoma have a poor survival rate, despite chemotherapy and bone marrow transplantation therapy.[1,3,5] The median survival varies between 0 and 5 years, with rare complete remission.[1]

The neoplastic cells are classically surface $CD3^+$, readily differentiating HSTCL from NK-cell neoplasms. Herein, we describe a unique case of de novo surface $CD3^-$ HSTCL and the challenge of differentiating it from NK-cell neoplasms, particularly when only morphologic and immunophenotype findings are available for the diagnosis.

REPORT OF A CASE

An 18-year-old man with a history of asthma was seen at the emergency department with recurrent epistaxis, nausea, headache for 3 days, and unintentional 9.1-kg weight loss during 3 weeks. Clinical examination and computed tomography revealed an enlarged spleen measuring 25.1 cm craniocaudally. There was no lymphadenopathy. Laboratory test results showed severe thrombocytopenia (platelet count, 33/μL), mild anemia (hemoglobin level, 11.8 g/dL), and normal total white blood cell count (8.8×10^3/μL). Aspartate aminotransferase and lactate dehydrogenase levels were elevated at 67 U/L and 535 U/L, respectively. Review of the peripheral blood smear noted blastlike cells with finely dispersed chromatin, prominent nucleoli, and fine cytoplasmic azurophilic granules (Figure 1, A through C). The flow cytometric study of the peripheral blood showed that the circulating neoplastic cells (comprising about 40% of total white blood cells) seemed to be of NK-cell lineage with expression of CD45 (bright), CD2, CD56, CD11b, CD16 (partial), and cytoplasmic CD3, but the sample was negative for surface CD3, CD57, CD4, CD5, CD7, CD8, TCR, CD123, terminal deoxynucleotidyl transferase, CD34, B-cell markers, or myeloid markers (Figure 2, A through F). A bone marrow aspirate and biopsy specimen revealed a hypocellular and moderately fibrotic marrow with sinusoidal infiltration of neoplastic cells best highlighted by cytoplasmic CD3 and CD56 on immunohistochemistry (Figure 1, D and E). Paired box protein PAX5, CD1a, terminal deoxynucleotidyl transferase, CD117, granzyme B, and CD34 were all negative in the neoplastic cells, ruling out B-cell involvement or acute lymphoid or myeloid leukemia.

The surface $CD3^-$ neoplastic cell expression of NK-cell markers, together with the leukemic presentation, led to an initial impression of an NK-cell neoplasm, in particular aggressive NK-cell leukemia (ANKL). However, the negative result of a heterophile antibody test for mononucleosis and undetectable serum EBV viral load rendered this diagnosis unlikely. Furthermore, molecular studies showed a clonal $\gamma\delta$ TCR gene rearrange-

Accepted for publication June 23, 2013.

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The authors have no relevant financial interest in the products or companies described in this article.

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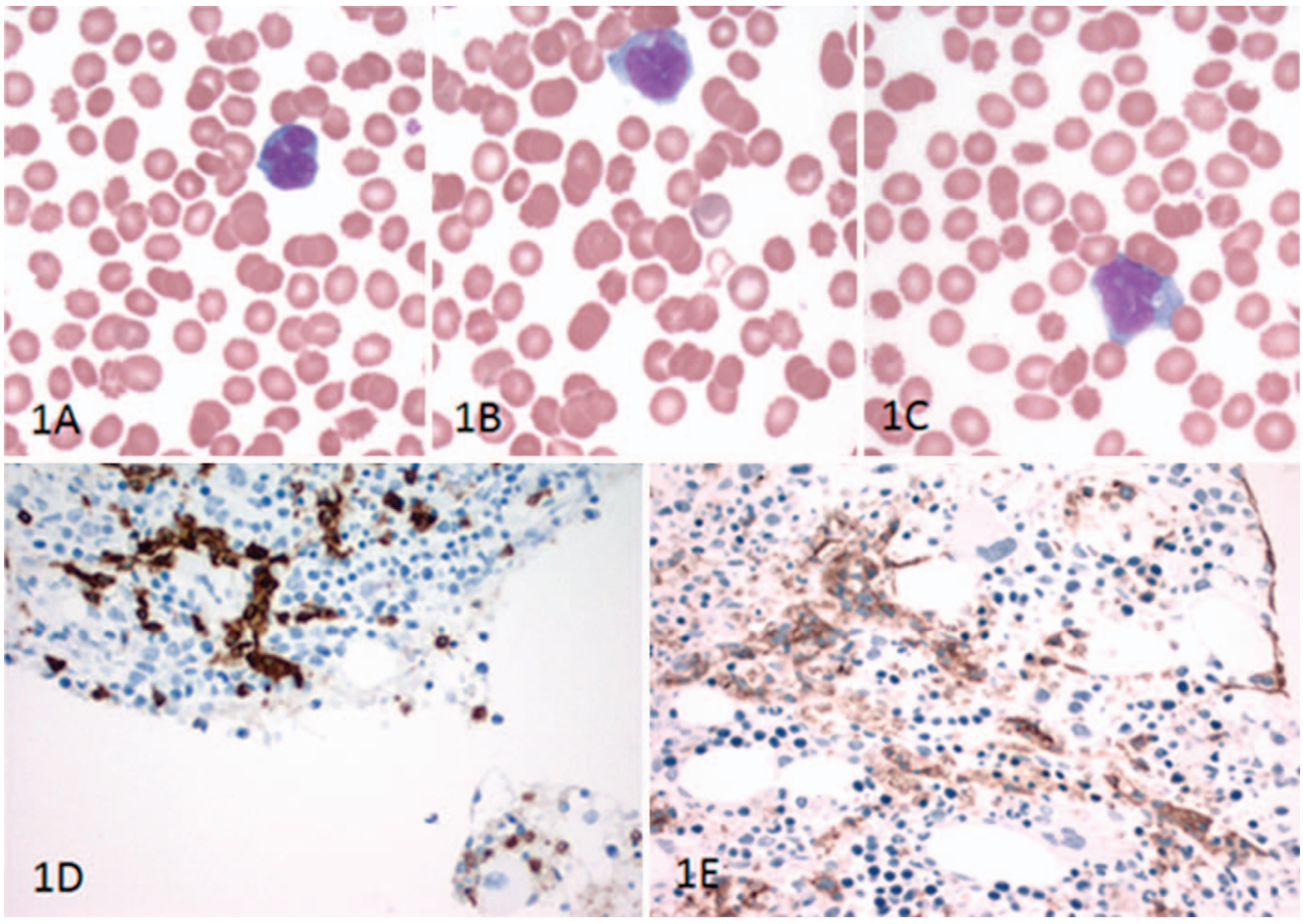

Figure 1. *A through C, Images of Giemsa and Wright–stained peripheral blood smears show blastlike cells with irregular nuclei, prominent nucleoli, and finely dispersed chromatin (original magnifications ×100). Immunostaining of the bone marrow core biopsy specimen shows neoplastic cells interspersed in the sinusoids with expression of cytoplasmic CD3 (D) and membranous CD56 (E) (original magnifications ×40).*

ment and cytogenetic abnormality of isochrome (7)(q10), indicating the diagnosis of surface CD3$^-$ HSTCL.

Our patient received methotrexate and prednisone, followed by the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) regimen. Shortly after chemotherapy treatment, the neoplastic population became surface CD3$^+$ and TCR–$\gamma\delta^+$ (Figure 3, A through D). Subsequently, he has received a combination of the chemotherapeutic drugs etoposide, methylprednisolone, high-dose cytarabine, and cisplatin (ESHAP therapy), followed by double umbilical cord blood transplantation. At 10 months after transplantation, the patient remained in complete remission with 100% donor engraftment.

COMMENT

This unique surface CD3$^-$ HSTCL case highlights the significant but often underappreciated overlap in clinical, morphologic, and immunophenotype findings between HSTCL and NK-cell neoplasms, in particular ANKL. For example, clinically ANKL also occurs in young and middle-aged adults, at a median age of 42 years.[6,7] Patients with ANKL can also initially be seen with hepatosplenomegaly and constitutional symptoms such as fever, liver dysfunction, and fatigue. Lymphadenopathy occurs only occasionally, but cytopenia is common.[7] In contrast to HSTCL, ANKL has a strong association with EBV, and some patients with ANKL may be seen with coagulopathies, hemophagocytic syndrome, and multiorgan failure because of the aggressive nature of the disease.[7]

Morphologically, HSTCL is characterized by homogeneous, mature, medium-sized postthymic T-lymphocytes infiltrating into the sinusoids and sinuses of the liver and spleen, respectively.[1,3] The white pulp of the spleen can be significantly reduced. Unlike in ANKL and our case, HSTCL cells are typified by medium-sized nuclei, loosely condensed chromatin, small inconspicuous nucleoli, and a rim of pale cytoplasm.[1,3] Overt leukemic presentation and lymphocytosis are rare at the initial examination; however, atypical lymphocytes can be identified in the peripheral blood smear of a few patients.[3] Bone marrow infiltration of the neoplastic lymphocytes with an intrasinusoidal pattern is present in approximately two-thirds of cases.[1] Erythrophagocytosis can be seen in areas invaded by the neoplastic infiltrate.[3]

In comparison, ANKL is characterized by the presence of circulating neoplastic NK cells, with a range of appearances from cells that are slightly larger than large granular lymphocytes to cells with irregular nuclear contour, enlarged nuclei, immature chromatin pattern, and prominent nucleoli.[7] Fine or coarse azurophilic granules are often seen in the slightly pale or basophilic cytoplasm. However, these characteristics were present in the circulating neoplastic cells of our patient with HSTCL. The morphologic impression of the neoplastic cells seen in the peripheral

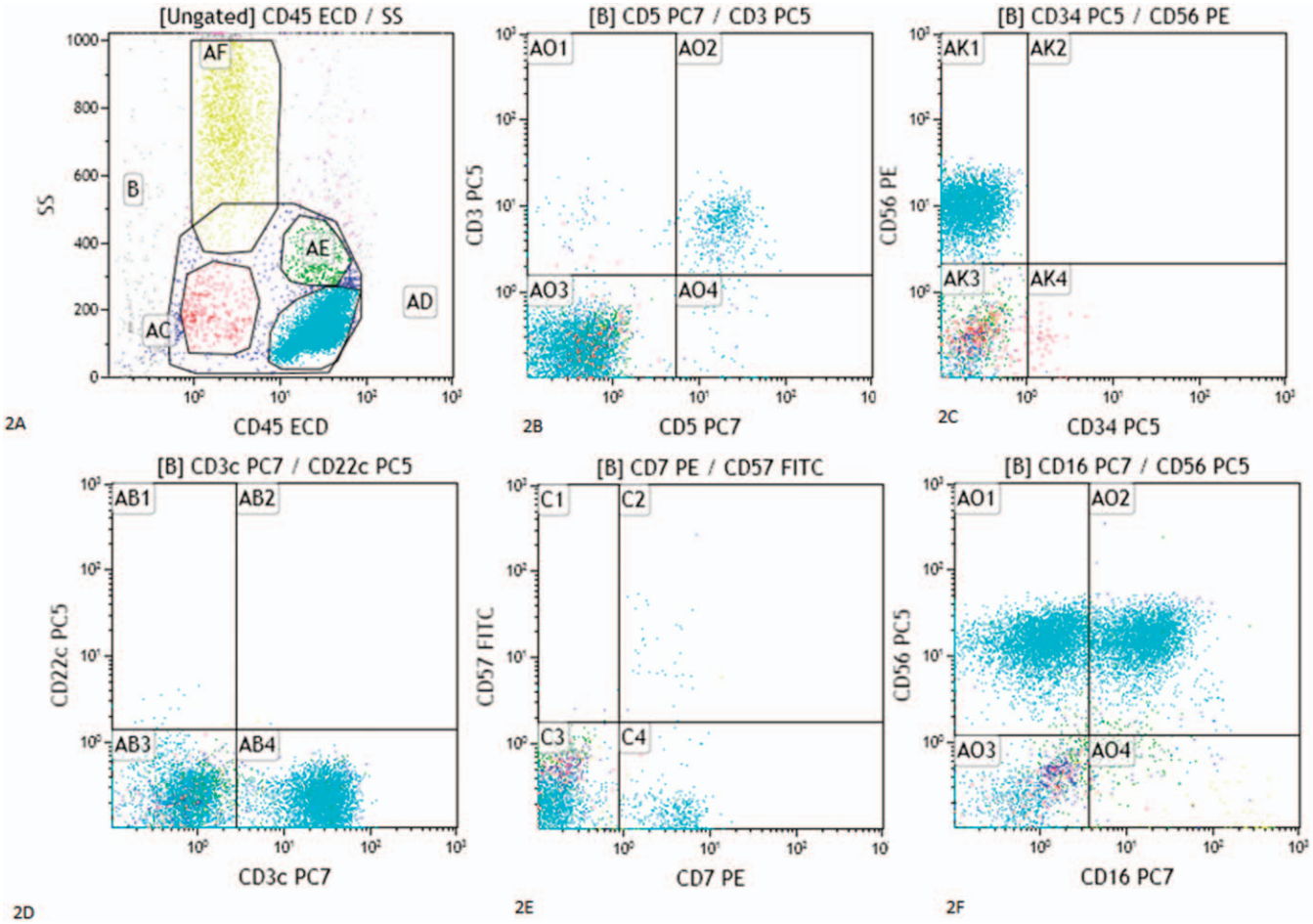

Figure 2. *Flow cytometry of the patient before treatment shows that most of the neoplastic cells are in cyan. A small population of surface CD3⁺ and CD5⁺ normal T cells is present in the background. The neoplastic cells have negative surface CD3 expression (B) and positive cytoplasmic CD3 expression (D). In addition, these cells express CD45 (bright), CD56, and CD16 (partial) but not CD57, CD5, CD7, CD22, or CD34 (A, C, E, and F).*

blood of our case initially favored ANKL over HSTCL. Varying degrees of bone marrow interstitial involvement by the neoplastic cell infiltrate with reactive histiocyte hemophagocytosis are commonly seen in ANKL.[8] Necrosis, apoptosis, and vascular involvement, including angioinvasion and angiodestruction, have been reported in ANKL tissue sections.[7] Similar to HSTCL, tumor cells of ANKL can also infiltrate the liver and spleen in the sinusoids and the red pulp (wall vessels), respectively.[7]

Immunophenotypically, the tumor cells of HSTCL usually express surface CD3, CD2, and CD7 and lack CD4, CD5, and CD8, with 15% of cases being $CD4^-/CD8^+$. The NK-cell–associated markers CD11b, CD16, and CD56 are variably expressed but are estimated to be present in 60% to 70% of all cases. CD57 is usually negative.[3] Cytotoxic granule-associated protein and granzyme M are usually expressed in the cells, while granzyme B and perforin are often negative.[3] Terminal deoxynucleotidyl transferase is negative and is useful in differentiating HSTCL from T-lymphoblastic lymphoma, which is characteristically $CD4^-/CD8^-$.

With the exception of lacking surface CD3 expression, ANKL cases can show an immunophenotype that is almost identical to that of HSTCL. The ANKL cases usually are $CD2^+$, cytoplasmic $CD3\varepsilon^+$, $CD56^+$, TIA-1^+, $CD4^-$, $CD5^-$, and $CD8^-$.[7] Similar to HSTCL, CD11b and CD16 show variable expression, while CD57 is usually negative.[8,9] Positive granzyme B and perforin expression and germline configuration of the TCR in ANKL are features that are helpful in distinguishing these 2 entities.[7]

Our case demonstrated significant morphologic and immunologic overlap between HSTCL and NK-cell neoplasms. The circulating neoplastic cells of this case were blastlike with large irregular nuclear contour and contained scant azurophilic granules, findings commonly seen in ANKL. Also similar to NK cells, the cells lacked surface CD3 expression and expressed the NK-cell–associated markers CD56, CD16, and CD11B. However, a diagnosis of $CD3^-$ HSTCL was rendered by the lack of EBV infection, positive TCR gene rearrangement, classic sinusoidal infiltrates of bone marrow, and characteristic cytogenetic finding of isochrome 7q and trisomy 8. The absence of EBV infection also excluded the possibility of systemic EBV^+ T/NK-cell lymphoproliferative disease of childhood, an entity that is more prevalent in Asia and may also be seen with hepatosplenomegaly and sinusoidal marrow infiltrates.[10] The negative expression of CD34, CD117, and terminal deoxynucleotidyl transferase ruled out the diagnosis of myeloid or lymphoid leukemia, which may lead to the circulating blastlike cells observed. The acute clinical course argued against the diagnosis of chronic lymphoproliferative disorder of NK cells. The lack of extranodal site involvement

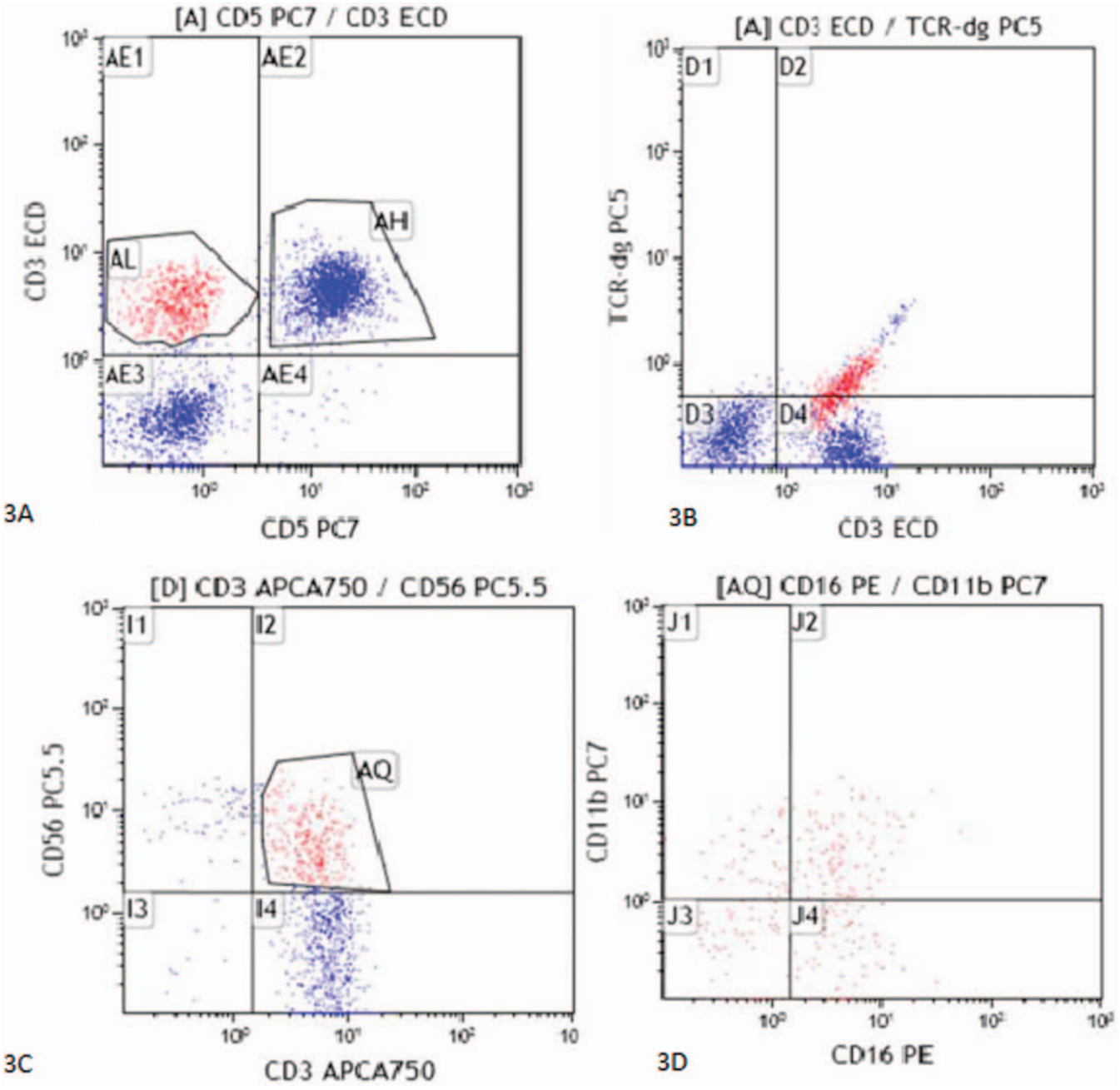

Figure 3. *Flow cytometry of the patient after treatment shows that the residual neoplastic cells highlighted in red have become surface CD3⁺. The neoplastic cells also express CD56 (C), negative T-cell receptor delta/gamma (TCR-dg) (B), and CD11b and CD16 (D) but not CD5 (A). The cells in blue are normal T cells present in the background (CD3⁺, CD5⁺, and CD56⁻).*

and EBV infection did not support the possibility of the nasal type of extranodal T/NK-cell lymphoma. Although biopsy of the liver and spleen was not performed in this case, with the exception of lacking surface CD3 expression, the clinical, morphologic, immunophenotype, cytogenetic, and molecular findings are all classic for HSTCL and ruled out other types of T-cell lymphoma.

Surface CD3⁻ HSTCL at initial presentation has not been previously reported to the best of our knowledge. There have been case reports of both blastic transformation of the neoplastic cells and the loss of surface CD3 marker in a few cases of HSTCL at the time of relapse.[11] CD3 is first expressed in the cytoplasm of the prothymocytes. As the T cells mature, CD3 is expressed on the surface membrane and forms a complex with TCR, which is important for activation of the T cells (ie, transducing stimulatory signals after antigen-specific recognition). The blastlike morphology of circulating cells in our case and the larger cells and loss of surface CD3 expression observed at presentation of relapse reported by others suggest that the absence of surface CD3 likely represents a blastic phase in the development of HSTCL.[5] The exact clinical significance of this type of presentation has yet to be determined because of the limited case reports at this time.

In conclusion, surface CD3⁻ HSTCL with blastlike morphology is a novel variant of HSTCL. The pathologist

should be aware of this entity when considering a diagnosis of T/NK-cell neoplasm. This case highlights the overlap between HSTCL and NK-cell neoplasms and the diagnostic challenges in identifying surface $CD3^-$ HSTCL. It is crucial to integrate clinical, morphologic, immunophenotype, molecular, and cytogenetic findings to reach an adequate diagnosis.

We thank Gary Pearl, MD, PhD, for reviewing and editing the article and Li Ge, MD, for formatting the figures.

References

1. Alsohaibani F, Abdulla MA, Fagih MM. Hepatosplenic T-cell lymphoma. *Indian J Hematol Blood Transfus*. 2011;27(1):39–42.

2. Beigel F, Jurgens M, Tillack C, et al. Hepatosplenic T-cell lymphoma in a patient with Crohn's disease. *Nat Rev Gastroenterol Hepatol*. 2009;6(7):433–436.

3. Vega F, Medeiros LJ, Bueso-Ramos C, et al. Hepatosplenic gamma/delta T-cell lymphoma in bone marrow: a sinusoidal neoplasm with blastic cytologic features. *Am J Clin Pathol*. 2001;116(3):410–419.

4. Steurer M, Stauder R, Grünewald K, et al. Hepatosplenic -T-cell lymphoma with leukemic course after renal transplantation. *Hum Pathol*. 2002;33(2):253–258.

5. Weidmann E. Hepatosplenic T cell lymphoma: a review on 45 cases since the first report describing the disease as a distinct lymphoma entity in 1990. *Leukemia*. 2000;14(6):991–997.

6. Song SY, Kim WS, Ko YH, et al. Aggressive natural killer cell leukemia: clinical features and treatment outcome. *Haematologica*. 2002;87(12):1343–1345.

7. Gao LM, Liu WP, Yang QP, et al. Aggressive natural killer-cell leukemia with jaundice and spontaneous splenic rupture: a case report and review of the literature. *Diagn Pathol*. 2013;8:43. http://www.ncbi.nlm.nih.gov/pubmed/?term=Aggressive+natural+killer-cell+leukemia+with+jaundice+and+spontaneous+splenic+rupture%3A+a+case+report+and+review+of+the+literature. Accessed July 1, 2013.

8. Ryder J, Wang X, Bao L, et al. Aggressive natural killer cell leukemia: report of a Chinese series and review of the literature. *Int J Hematol*. 2007;85(1):18–25.

9. Suzuki R, Suzumiya J, Nakamura S, et al. Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. *Leukemia*. 2004;18(4):763–770.

10. Kimura H, Ito Y, Kawabe S, et al. Epstein-Barr virus (EBV)–associated T/NK lymphoproliferative disease in nonimmunocompromised hosts: prospective analysis of 108 cases. *Blood*. 2012;119(3):673–686.

11. Khan WA, Yu L, Eisenbrey AB, et al. Hepatosplenic gamma/delta T-cell lymphoma in immunocompromised patients: report of two cases and review of literature. *Am J Clin Pathol*. 2001;116(1):41–50.