Reverse Warburg Effect in a Patient With Aggressive B-Cell Lymphoma: Is Lactic Acidosis a Paraneoplastic Syndrome?

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Seminars in Oncology

At times we encounter clinical problems for which there are no directly applicable evidence-based solutions, but we are compelled by circumstances to act. When doing so we rely on related evidence, general principles of best medical practice, and our experience. Each ``Current Clinical Practice" feature article in *Seminars in Oncology* describes such a challenging presentation and offers treatment approaches from selected specialists. We invite readers' comments and questions, which, with your approval, will be published in subsequent issues of the Journal. It is hoped that sharing our views and experiences will better inform our management decisions when we next encounter similar challenging patients. Please send your comments on the articles, your challenging cases, and your treatment successes to me at dr.gjmorris@gmail.com. I look forward to a lively discussion.

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Lactic acidosis is defined as the presence of an elevated blood lactate level (>5 mmol/L) with acidosis (pH<7.3).[1] Lactate is generated as the end product of glycolysis in the cytoplasm, when pyruvate is not metabolized to carbon dioxide and water by oxidative phosphorylation (OXPHOS) in mitochondria. Type A lactic acidosis or acidosis due to anaerobic conditions is most commonly due to insufficient oxygen delivery to tissues, which inhibits OXPHOS. Common causes of type A lactic acidosis are ischemic bowel syndrome, sepsis, cardiogenic shock, and hypovolemia. On the other hand, type B lactic acidosis or acidosis, despite aerobic conditions, has been described in patients with normal oxygen delivery to tissues. Although rare, type B lactic acidosis has been reported in hematologic malignancies, human immunodeficiency virus infection, diabetes mellitus, liver disease, thiamine deficiency, and hereditary mitochondrial enzymatic deficiencies, as well as medication side effects.[2]

The majority of cancer-induced lactic acidosis cases have been reported in patients with aggressive lymphomas.[3–7] Lactic acidosis is a life-threatening condition in critically ill patients, with a mortality rate of >50%.[8,9] In cancer patients, lactic acidosis has an even worse prognosis, with a mortality rate >80%.[3,10,11] Currently, the only available treatment for lactic acidosis is reversal of the offending cause.

The traditional view of cancer metabolism, also known as the Warburg effect, is that cancer cells have impaired mitochondrial respiration even in the presence of oxygen. They therefore metabolize glucose mainly to lactate (aerobic glycolysis, type B lactate generation). The Warburg effect is energetically very inefficient since it only generates 2 to 4 moles of adenosine triphosphate (ATP) per mole of glucose, compared to 36 moles of ATP per mole of glucose with OXPHOS metabolism. The reason why cancer cells would utilize this inefficient metabolic strategy is unknown, since they require large amounts of ATP to proliferate.[12] Our knowledge of tumor metabolism is critically important for the modern management of cancers and ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) scans are used for the diagnosis, staging, and prognosis of aggressive lymphomas.

Human tumor tissues compared to healthy tissues show on average a 10-fold higher rate of glucose uptake and glycolysis.[13] PET scans using FDG as a radiotracer are

used to follow whole body glucose uptake and metabolism. Deoxyglucose is uptaken into highly glycolytic cells, where it remains trapped because it cannot undergo further catabolism.[14] FDG will emit positrons, providing an image of glycolytic tumors.[15]

Aggressive lymphomas are among the most PET-avid tumors.[16] However, FDG-PET scans cannot identify the glycolytic cell types within a given tumor since lymphomas are composed not only of cancer cells but also of a stroma with different supporting cells such as fibroblasts, macrophages, noncancerous lymphocytes, and endothelial cells.[17] In fact, in some lymphoma subtypes, the tumor glucose avidity is likely due to stromal or reactive cells. Hodgkin lymphomas are highly FDG-PET–positive, yet the majority of the cells within the tumor are reactive stroma with less than 1% of cells being neoplastic.[18] Therefore, the high glucose avidity of these tumors is likely due to the stromal compartment. Moreover, lymph nodes originally involved by lymphoma remain glucose-avid by FDG-PET weeks after treatment even though no evidence of lymphoma is found, indicating that FDG-PET avidity is due to the stroma.[16] In addition, tissues surrounding lymphomas are frequently FDG-PET–positive, despite not being involved by lymphoma cells.[16] These examples demonstrate that, in some instances, it is the stroma or tumor microenvironment that is FDG-PET–positive instead of the lymphoma cells within tumors.

Recently, tumor bioenergetic studies have demonstrated that stromal cells have a glycolytic or lipolytic metabolism, while cancer cells extract catabolites from the stroma and have increased mitochondrial OXPHOS metabolism with low glucose uptake and glycolysis.[19–24] This new paradigm of stromal–cancer cell metabolic coupling has been termed the "reverse Warburg effect" since aerobic glycolysis occurs in the stroma and allows cancer cells to be very efficient energetically with high ATP production, hence solving the energetic paradox of the Warburg effect.[25]

Stromal–cancer cell metabolic coupling is mediated by oxidative stress.[26] Cancer cell–initiated oxidative stress leads to mitochondrial damage and autophagy (self-eating) in stromal cells.[27] Mitochondrial damage and autophagy in turn induce stromal glycolysis and catabolite efflux with lactate release from the stromal cells.[20] Cancer cells are protected from the deleterious effects of oxidative stress by upregulation of endogenous antioxidants. Importantly, cancer cells uptake stromal-released catabolites, such as lactic acid, glutamine and fatty acids, to sustain their high OXPHOS metabolism.[23,28] The increased mitochondrial activity of cancer cells in proximity to glycolytic stroma has been measured both by functional assays in vitro and by assaying complex I and IV mitochondrial OXPHOS activity in frozen tumor sections.[19–21,29] In this regard, lactic acid is no longer considered a waste product of glycolytic metabolism, but is instead an oncometabolite that promotes mitochondrial metabolism and tumor growth.[30]

Under these conditions of metabolic coupling, cancer cells show profound DNA damage, genomic instability, and resistance to apoptosis.[19,20,31] Mitochondrial health in cancer cells correlates with drug resistance.[32] Also, inhibiting mitochondrial OXPHOS induces cancer cell death under conditions of stromal–cancer metabolic coupling.[33,34] Pharmacological inhibition of autophagy impairs mitochondrial biogenesis in cancer cells and induces apoptosis by blocking the flow of catabolites to cancer cells.[28] This observation highlights the importance of stromal autophagy in promoting cancer cell growth.

Here, a patient with diffuse large B-cell lymphoma (DLBCL) and type B lactic acidosis was studied to understand the role of cancer cell and stromal cell compartment-specific metabolism in tumorigenesis and to determine the root cause of the lactic acidosis.

CASE PRESENTATION

A 50-year-old man with a history of hepatitis C virus (HCV) infection diagnosed 5 years ago complicated with advanced liver disease in addition to hypertension, diabetes, and tobacco use presented to the emergency room complaining of increasing abdominal girth and lower extremity edema over the past 10 days. A review of systems revealed night sweats, mild shortness of breath, and reduced urinary output without fever, chills, pruritus, or weight loss. The patient on presentation was afebrile, had a heart rate of 107 beats per minute, respiratory rate of 24 breaths per minute, blood pressure of 133/72 mm Hg, and had an oxygen saturation of 94% on room air. Initial physical examination revealed conjunctival icterus, normal cardiac and pulmonary auscultation, abdominal distention with palpable splenomegaly and dullness to percussion, palpable bilateral axillary lymphadenopathy, bilateral lower extremity edema, and a normal neurological exam without asterixis.

The patient was admitted with a presumptive diagnosis of decompensated liver failure and cirrhosis. Blood laboratory tests revealed lactic acid 14.4 mm/L (normal value [NV]: 0.5–2.2 mm/L) and bicarbonate (HCO_3) 15 mEq/L (NV: 24–32 mEq/L). Tumor lysis panel showed lactate deydrogenase (LDH) 559 IU/L (NV: 125–240 IU/L), LDH1 15% (NV: 19%–38%), LDH2 31% (NV: 30%–43%), LDH3 25% (NV: 16%–26%), LDH4 17% (NV: 3%–12%), LDH5 13%

(NV: 3%–14%), uric acid 13.3 mg/dL (NV: 3.5–9.0 mg/dL), potassium 5.5 mEq/L (NV: 3.5–5 mEq/L), calcium 9.9 mg/dL (NV: 8.5–10.5 mg/dL), and phosphate 4.3 mg/dL (NV: 2.4–4.5 mg/dL). Additional blood work showed creatinine 1.2 mg/dL, total bilirubin 2.1 mg/dL, direct bilirubin 0.6 mg/dL, albumin 2.8 g/dL, AST 124 IU/L, and ALT 47 IU/L. Coagulation panel results were prothrombin time PT 17.9 seconds, international normalized ratio (INR) 1.52, and partial thromboplastin time (PTT) 32 seconds. White blood cell (WBC) count was 4.4 10^9/L, hemoglobin (Hb) 11 g/dL, heamtocrit (Hct) 32.6%, and platelet count 53,000 10^9/L.

Paracentesis revealed a serum ascites albumin gradient (SAAG) of 2.1, which is consistent with portal hypertension. Cytology of ascitic fluid showed few neutrophils but abundant atypical cells suspicious for malignancy with occasional mitotic figures. Flow cytometry of the ascitic fluid revealed a population of lymphocytes that were $CD19^+$, $CD20^+$, $CD22^+$, $CD5^-$, $CD10^-$, $CD23^-$, and $CD25^+$. These lymphocytes were surface lambda light chain–restricted. A computed tomography (CT) with intravenous contrast of the abdomen showed a cirrhotic liver, innumerable diffuse hypo-enhancing splenic masses, abdominal lymphadenopathy, and peritoneal implants suggestive of lymphoma and peritoneal lymphomatosis. A chest computed tomography (CT) also showed enlarged axillary, mediastinal, and pericardiophrenic lymph nodes.

Due to the high suspicion of lymphoma, the patient had a bone marrow biopsy and an excisional lymph node biopsy of an enlarged right axillary lymph node. The lymph node biopsy confirmed the diagnosis of DLBCL. The final diagnosis was DLBCL stage IVB and type B lactic acidosis with evidence of laboratory tumor lysis. His Revised International Prognosis Index (R-IPI) was 4, which is associated with a poor outcome with an estimated overall survival (OS) of 55% at 5 years.

On day 5 of hospitalization, the patient was started on chemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) with a 50% dose reduction in doxorubicin (25 mg/m^2) and a 25% reduction in cyclophosphamide (560 mg/m^2) due to hepatic dysfunction, with myeloid growth factor support. He was initiated on standard tumor lysis treatment with intravenous fluids and rasburicase. He received the first two cycles of chemotherapy as an inpatient with significant clinical and laboratory improvement. Notably his lactic acid normalized 8 days after the initiation of chemotherapy; however, his LDH had not normalized 232 days after initial chemotherapy treatment. A timeline of laboratory values for acidosis is presented in Table 1. The patient completed a total of six cycles of R-CHOP and the most recent PET/CT scan showed no evidence of disease.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board at Thomas Jefferson University Hospital and informed consent was obtained from the patient. Pathological analyses were performed on ascitic fluid, a bone marrow biopsy from the right posterior superior iliac crest, and a right axillary excisional lymph node biopsy sample. The lymph node biopsy was fixed in 10% neutral buffered formalin and embedded in paraffin for hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining. The bone marrow biopsy was decalcified and stained for H&E and IHC analysis. The ascitic fluid was evaluated by cytology and flow cytometry. The bone marrow aspirate was evaluated by Wright Giemsa staining, flow cytometry, karyotyping and DNA fluorescence in situ hybridization (FISH) analysis using the following probes: IgH, BCL-6, BCL-2, and c-MYC. The streptavidin–horse radish peroxidase method was used for IHC staining as previously described.[35] Primary antibodies used were CD20 (Ventana, Oro Valley, AZ, 760-2531), BCL-2 (Ventana, 790-4604), BCL-6 (Ventana, 760-4241), Ki-67 (Ventana, 790-4286), CD34 (Ventana,

Table 1. Timeline of Laboratory Blood Values

	Day										
Parameter	1	3	5*	6	7	8	10	13	19	32	237
pH	7.29		7.25	7.34	7.4						
HCO_3 (mEq/L)	15	14	11	15	21	22	26	30	26	29	29
Anion gap (mEq/L)	17	19	24	19	10	9	5	5	6	12	10
LDH (IU/L)	559	549	616	648	664	882	557	468	347	291	250
Lactic acid (mmol/L)	14.4		18.4	15.9	6.7	3.7	3.1	2.2			2.2

* Denotes first day of R-CHOP treatment.

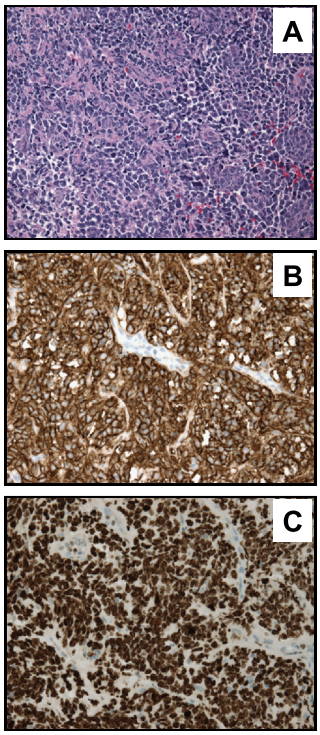

Figure 1. Pathological study of axillary lymph node infiltrated by DLBCL. Formalin-fixed paraffin-embeded sections were analyzed. (A) Hematoxylin and eosin (H&E). Note the diffuse infiltration of large atypical cells with effacement of the lymph node architecture. A representative image is shown. (B) CD20. Sections were immunostained for CD20 (brown) and counterstained with hematoxylin (blue). Note the significant infiltration of the lymph node by B-cell lymphoma cells with high CD20 expression on the cell membrane. A representative image is shown. (C) Ki67. Sections were immunostained for Ki67 (brown) and counterstained with hematoxylin (blue). Note the high proliferation rates of the tumor cells (>95%). A representative image is shown. For all panels, original magnification, 40x.

790-2927), TOMM20 (Santa Cruz, Santa Cruz, CA, sc-17764), LDHB (Sigma, St Louis, MO, AV48210), PKM1 (Proteintech, Chicago, IL, 15821-AP), PKM2 (Proteintech, 15821-1-AP), cathepsin B (Santa Cruz, sc-13985), and beta galactosidase (Abcam, Cambridge, MA, ab96239). MCT4 and MCT1 antibodies were kindly provided by Dr Nancy Philp and have been extensively characterized.[36]

RESULTS

A right axillary lymph node biopsy was diagnostic for DLBCL, germinal center type (GC). The lymph node architecture was effaced by atypical lymphocytes with large nuclei as seen by H&E staining (Figure 1A). Staining for CD20, CD3, BCL2, BCL6, CD10, MUM1, kappa and lambda light chains, EBER, reticulin, and Ki67 was performed. The malignant cells had a GC phenotype. They were lambda light chain–restricted, $BCL6^{+}$ (Figure 7), and $CD20^{+}$ (Figure 1B). The malignant cells were negative for CD10, CD3, BCL2, and MUM1. Epstein-Barr staining by Epstein-Barr–encoded RNA (EBER) in situ hybridization was negative. The malignant cells had very high proliferation rates, as shown by positive Ki-67 staining in 95% of cells (Figure 1C). DNA FISH of the axillary lymph node for rearrangement of BCL6 (3q27), c-MYC (8q24), and BCL2 (18q21) were performed and did not show rearrangement, although 20% of cells had monosomy of BCL2.

Bone marrow involvement by lymphoma was not evident by morphology but was detected by DNA FISH with BCL6 rearrangement. FISH showed four fused signals with two additional signals smaller in size suggesting a derivative or rearranged chromosome at 3q27. The karyotype was normal. Thus, from the FISH analysis of the lymph node and bone marrow biopsies, it appears that the patient has at least three cancer clones. There was also an increase of fibrosis in the bone marrow, as seen by reticulin staining, without evidence of morphological lymphoma involvement (data not shown).

In order to assess which type of cells within the tumor were generating the lactic acid, we analyzed the lymph node biopsy with a panel of metabolic markers of glycolysis, autophagy, and OXPHOS. Pyruvate kinase isoform 1 (PKM1) is one of the main enzymes involved in lactic acid generation. Lactate dehydrogenase-B (LDHB) catalyses the last step of glycolysis and converts pyruvate to lactic acid. Monocarboxylate transporter 4 (MCT4) is the main exporter of lactic acid out of cells. Interestingly, the expression of PKM1 (Figure 2A), PKM2 (Figure 2B), LDHB (Figure 2C), and MCT4 (Figure 2D) were largely confined to stromal cells and were nearly absent in cancer cells.

To further characterize the stromal metabolism, the lymph node biopsy was immunostained with cathepsin B and with beta galactosidase, which are markers of autophagy and senescence. Cathepsin B and beta galactosidase staining was found in the stroma and was absent in cancer cells with very similar staining patterns (Figure 3A and 3B).

To determine the relative contents of stroma and extracellular matrix, we stained the lymph node sample for reticulin and found that there was increased staining in both the lymph node (Figure 4A) and bone marrow biopsy (not shown) consistent with a reactive stroma and fibrosis. Morphologically, the bone marrow was negative for lymphoma, although, as previously stated, BCL6 was amplified by DNA FISH consistent with lymphoma bone marrow involvement. To further characterize whether some of the lymph node stromal staining was due to endothelial cells, we stained for the endothelial marker CD34. Blood vessels are visible (Figure 4B), although they show a different pattern and are less abundant than PKM1, LDHB, MCT4, and cathepsin B.

To determine whether cancer cells take up lactic acid generated by the stroma, the lymph node was immunostained with antibodies

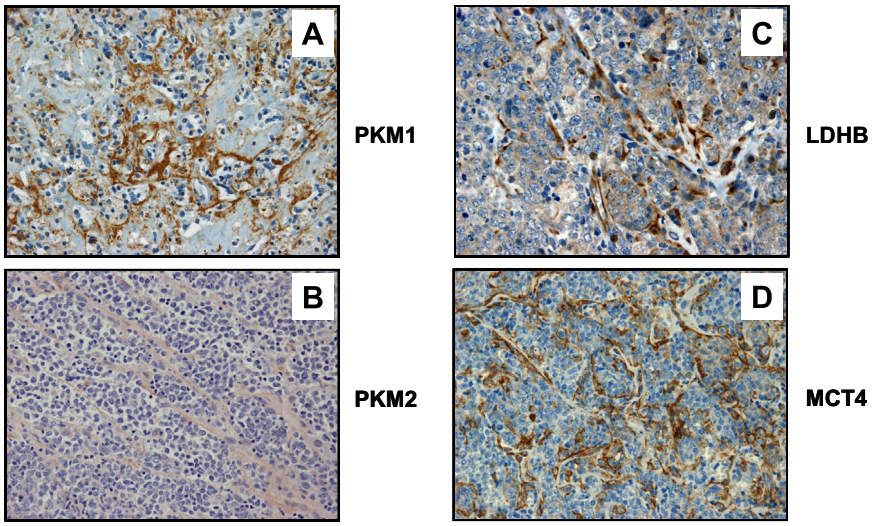

Figure 2. Lymphoma-associated stroma generates and secretes lactic acid. Paraffin-embedded sections of an axillary lymph node were immunostained with the indicated markers of glycolysis (brown) and counterstained with hematoxylin (blue). (A) PKM1. Note that PKM1, a marker of lactic acid generation, is highly expressed in the stromal cells surrounding the lymphoma cells, and largely absent from cancer cells. A representative image is shown. Original magnification, 40x. (B) PKM2. Note that PKM2, a marker of lactic acid generation and oxidative stress, is highly expressed in the stromal cells surrounding the lymphoma cells, and largely absent from cancer cells. A representative image is shown. Original magnification, 40x. (C) LDHB. Note that LDHB, a marker of lactic acid generation, is preferentially expressed in the stromal cells surrounding the lymphoma cells, and excluded from cancer cells. A representative image is shown. Original magnification, 60x. (D) MCT4. Note that the expression of MCT4, the transporter for lactic acid export, is largely confined in the lymphoma-associated stromal cells, and excluded from the cancer cells. A representative image is shown. Original magnification, 40x.

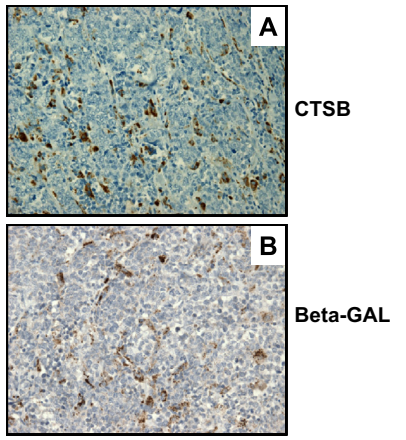

Figure 3. Lymphoma-associated stroma display high expression of cathepsin B and beta galactosidase markers of autophagy and senescence. Paraffin-embedded sections of an axillary lymph node were stained as indicated. (A) Cathepsin B. Note that cathepsin B (brown), a marker of autophagy, is highly expressed in the stromal compartment surrounding the lymphoma cells. A representative image is shown. Original magnification, 40x. (B) Beta galactosidase. Note that beta galactosidase (brown), a marker of senescence and autophagy, is highly expressed in the stromal compartment surrounding the lymphoma cells. A representative image is shown. Original magnification, 40x.

against MCT1, the transporter for lactate import. High MCT1 expression was found in a subset of cancer cells but largely absent in the stroma (Figure 5). The cancer cells did not show evidence of increased lactic acid generation and glycolysis, as shown by lack of PKM1, LDHB, and MCT4 expression. To investigate if cancer cells have a mitochondrial OXPHOS metabolism instead of a glycolytic metabolism, the lymph node was immunostained for mitochondrial outer membrane translocase 20 (TOMM20) and BCL6. TOMM20 is a marker of mitochondrial mass and activity, and BCL6 is a marker of increased mitochondrial membrane potential, and decreased apoptosis. High staining for BCL6 and TOMM20 was found in the cancer cells while staining in the stroma was absent (Figures 6 and 7), suggesting that lymphoma cells have active and healthy mitochondria.

DISCUSSION

Metabolic Coupling: Glycolytic Stroma and Anabolic Lymphoma Cells

We present a patient with a high-grade lymphoma and lactic acidosis, and we show that the lactic acidosis is due to the cancer stroma. We also present evidence of metabolic coupling between stromal cells and lymphoma cells with lactic acid secretion from stromal cells and uptake in lymphoma cells, as demonstrated by the expression patterns of glycolytic and mitochondrial markers (Figure 8A and B).

It is well known that in physiological conditions, lactic acid shuttles allow the metabolic coupling between different noncancerous cells. MCT4 is present at high concentrations in fast-twitch or white muscle fibers, which are glycolytic, and excrete lactic acid to be taken up via MCT1 by slow-twitch or red muscle fibers.[37–39] The muscle–liver lactic acid shuttle (Cori cycle) and the muscle–kidney lactic acid shuttle allow lactic acid uptake by the liver and kidney.[24] A lactic acid shuttle is also found between cumulus granulosa cells, which are glycolytic and express MCT4, and preimplantation oocytes, which express MCT1 and utilize lactic acid for mitochondrial OXPHOS.[40,41] Although lactic acid shuttles are the best characterized metabolite

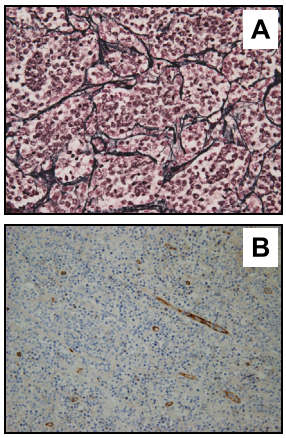

Figure 4. Lymphoma-associated stroma show increased fibrosis, with moderate blood vessel infiltration. Paraffin-embedded sections of an axillary lymph node were immunostained with the indicated antibodies. (A) Reticulin. Note that reticulin, a marker of increased reactive stroma, is highly expressed in the involved lymph node. A representative image is shown. Original magnification, 40x. (B) CD34. Immunostaining with CD34, an endothelial cell marker, shows a moderate amount of blood vessels. $CD34^{+}$ cells are fewer than cells positive for glycolysis markers (PKM1, LDHB, and MCT4), suggesting that increased glycolysis is more pronounced than angiogenesis infiltration. A representative image is shown. Original magnification, 40x.

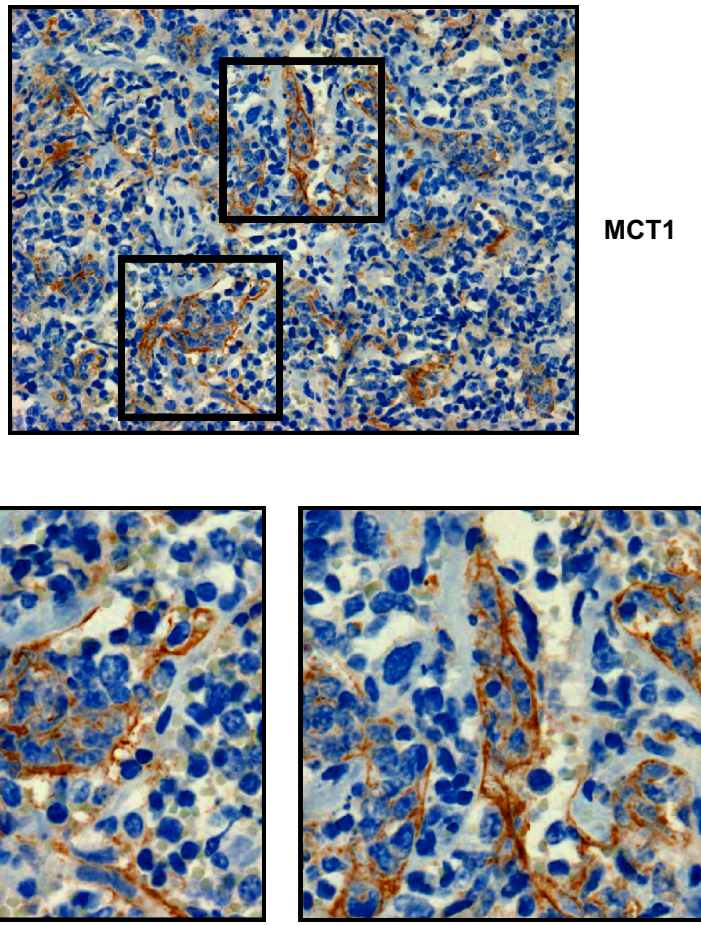

Figure 5. Lymphoma cancer cells have high lactic acid uptake. To visualize lactic acid uptake, lymph node sections were immunostained with MCT1, a transporter for lactic acid import. Note that MCT1 is highly expressed in a subset of lymphoma cancer cells. A representative image is shown, and two higher power magnifications are shown to better visualize MCT1 staining at the plasma membrane of lymphoma cells. Original magnification, 40x.

shuttles, other types exist between adipocytes and muscle cells to transfer fatty acids.[42]

Metabolic coupling between cells within a tumor has been described only recently, and MCTs allow lactic acid transfer from hypoxic to well-oxygenated cells in experimental models.[43] In human breast cancer, metabolic coupling with high MCT4 expression in the stroma and high MCT1 expression in cancer cells has been reported.[44] Finally, metabolic coupling has been found between ovarian cancer cells and omental cells in metastatic ovarian cancer via the fatty acid transporter FABP4, with fatty acid transfer from adipocytes to ovarian cancer cells and increased OXPHOS in ovarian cancer cells.[21]

Here, we describe for the first time a patient with a stromal–lymphoma lactic acid shuttle (Figure 8A). Our data indicate that in our patient with lymphoma and lactic acidosis, the high lactic acid levels generated by the stroma support tumor growth. LDH expression has been shown to correlate with glycolysis and impairment in OXPHOS.[45] LDH expression was studied in this patient's pathologic lymph node to determine which cell type generates the large amounts of lactic acid measured in whole blood. More than 70% of this patient's LDH subunits in whole blood were LDHB. Hence we focused on this subunit. LDHB was expressed at high levels in the stromal cells and absent in the cancer cells, clearly indicating that the generation of lactic acid occurs in the stroma.

Pyruvate kinase is a glycolytic enzyme that bidirectionally converts phosphoenolpyruvate to pyruvate.[46] PKM1 overexpression in stromal cells has been shown to generate high levels of lactic acid, and to support tumor growth in experimental mouse models.[47] There is now evidence that aggressive cancer cells suppress PKM2 activity to generate an antioxidant response.[48] Our patient had high LDHB, PKM1, and PKM2 expression in the stromal cells, consistent with high lactic acid generation, with absence of LDHB, PKM1, and PKM2 expression in

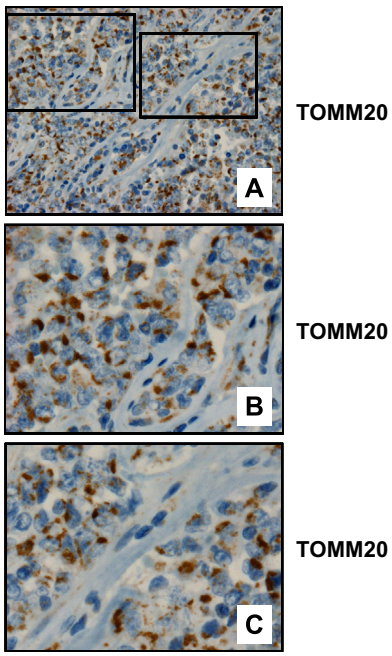

Figure 6. Lymphoma cancer cells have high mitochondrial metabolism. Paraffin-embedded sections of an axillary lymph node were immunostained with TOMM20 (brown), a mitochondrial marker and counterstained with hematoxylin (blue). Note that TOMM20 is highly expressed in the lymphoma cancer cells and is absent in the surrounding stromal cells. A representative image is shown (A), and two higher power magnifications of the insets are shown (B, C) to better visualize the elevated TOMM20 staining in the lymphoma cells and lack of staining in the stromal cells. Original magnification, 60x.

lymphoma cells, consistent with reduced glycolysis and an antioxidant response (Figure 8A and B).

We also show that lymphoma-associated stromal cells have increased expression of MCT4, the main transporter involved in lactic acid efflux out of cells. MCT4 is a target gene of HIF-1alpha, the main transcription factor involved in inducing glycolysis.[45,49] Importantly, MCT4 expression correlates with glycolysis and reduced OXPHOS.[37] Thus, elevated MCT4 expression further indicates that lymphoma-associated stromal cells are highly glycolytic.

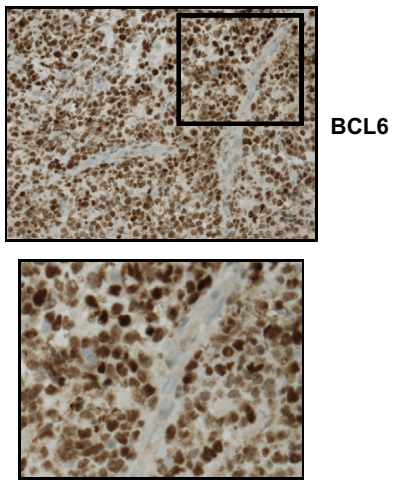

Figure 7. BCL6, a marker for mitochondrial metabolism and GC B-cell origin, is preferentially expressed in the lymphoma cells. Paraffin-embedded sections of an axillary lymph node were immunostained for BCL6 (brown) and counterstained with hematoxylin (blue). Note that BCL6 is highly expressed in the lymphoma cancer cells and is absent in the surrounding stromal cells. A representative image is shown, with a higher power magnification of the boxed area. Original magnification, 40x.

Healthy mitochondria have two main functions in cells. The first function is to serve as the cellular powerhouse for energy generation.[50] The second function is to protect against apoptosis by maintaining a high mitochondrial membrane potential, and preventing intramitochondrial content release and the apoptosis pathway.[51]

We characterized mitochondrial metabolism and apoptosis mediators in this patient. High levels of BCL6 and TOMM20 were found in the patient's lymphoma cancer cells. BCL6 increases mitochondrial metabolism and membrane potential, decreases oxidative stress and apoptosis rates, and increases proliferation in GC-derived lymphomas.[52–54] TOMM20 is a component of the outer mitochondrial membrane whose expression correlates with mitochondrial OXPHOS activity.[55,56] Thus, the finding that the patient's lymphoma cells express BCL6 and TOMM20 is consistent with high mitochondrial metabolism and resistance to apoptosis (Figure 8A and B). Conversely, stromal cells have high expression of the autophagy and senescence markers cathepsin B and beta galactosidase. High autophagy and senescence is found in highly catabolic cells with mitochondrial dysfunction and high glycolysis.[57,58]

Importantly, the lymphoma cells had also high levels of MCT1, the transporter for the uptake of lactic acid, which can serve as a substrate of mitochondrial metabolism and fuel OXPHOS. Consistently, MCT1 expression correlates with OXPHOS mitochondrial metabolism.[59,60] Thus, healthy mitochondria in the lymphoma cells are likely to be driving tumorigenesis and cancer progression (Figure 8A and B).

Stromal-Derived LDH as a Potential Marker of Genetic Instability and Secondary Malignancies

DLBCL is the most common type of lymphoma.[61] Approximately 50% of these patients will relapse.[62] Hence, a better understanding of DLBCL pathophysiology and new prognostic strategies to improve outcomes are urgently needed. LDH blood levels at diagnosis is one of the most important predictors of outcome and is part of the most commonly used prognostic models for lymphomas.[63–65] To our knowledge, this is the first study to analyze the metabolism of the tumor stroma in an aggressive human lymphoma. The elevated blood LDH levels in this patient are being generated by stromal cells, and it is likely that elevated LDH levels may have different prognostic significance if derived from stromal or cancer cells. It is noteworthy that this patient had different genetic abnormalities in his lymph node and bone marrow biopsy. This suggests that a

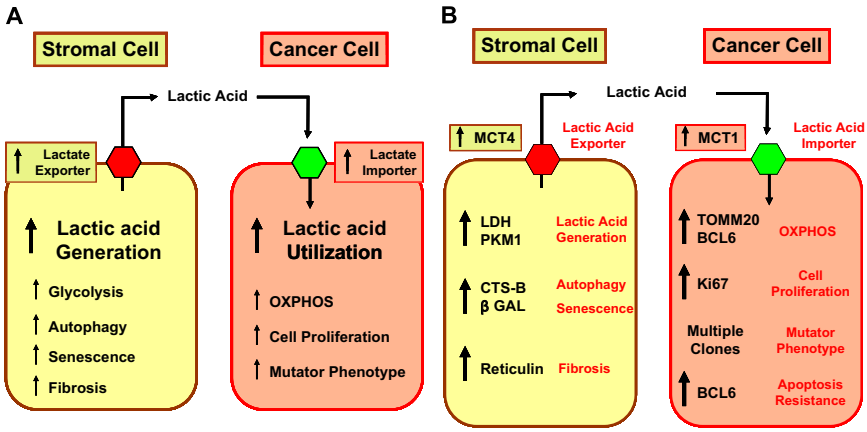

Figure 8. Schematic representation of the paraneoplastic lactic acidosis and stromal–cancer cell metabolic coupling. (A) Overall diagram of stromal-cancer cell metabolic coupling. Lymphoma-associated stromal cells have high fibrosis, autophagy, senescence, and glycolysis, which leads to the generation and excretion of lactic acid, via lactate transporter. Thus, lactic acidosis is due to the stromal cells. Conversely, cancer cells have high levels of transporters for lactic acid uptake, which allow for its utilization in mitochondrial OXPHOS metabolism. Cancer cells also have very proliferation rates and a mutator phenotype with multiple clones being detected. (B) Summary of the staining results in a patient with lymphoma and lactic acidosis. In the patient with lymphoma and lactic acidosis, lymphoma-associated stromal cells generate and secrete lactic acid, as seen by the high expression of LDHB and PKM1, which are markers of lactic acid generation, and high expression of MCT4 which is the main lactate exporter out of cells. The stromal cells are also highly catabolic with increased autophagy and senescence, as shown by the high expression of cathepsin B and beta galactosidase. Also, the tumor stroma is highly fibrotic with high reticulin deposition consistent with a reactive stroma. Cancer cells utilize lactic acid for their mitochondrial metabolism, as shown by high expression of MCT1, which is the main lactate importer into cells, high expression of TOMM20 and BCL6 whose expression correlates with mitochondrial OXPHOS metabolism and resistance to apoptosis. Lymphoma cells have high proliferation rates as measured by Ki-67 staining. Finally, multiple clones of cancer cells were detected by DNA FISH. Lactic acidosis due to the stroma and stromal–cancer cell metabolic coupling likely favor aggressive tumor development. Two issues of particular interest in the care of this patient are how to monitor and decrease the risk of recurrence or secondary malignancies.

transformed stroma leads to a mutator phenotype, whereby new mutations are acquired. It has been demonstrated that the stroma favors genetic instability and aneuploidy in cancer cells under conditions of metabolic coupling between cancer cells and stromal cells.[20] Thus, an elevated LDH level due to the stroma may be a marker of genetic instability. Further studies are warranted to evaluate this point.

The only treatment that has been shown to be effective in managing lymphoma associated lactic acidosis is the treatment of the lymphoma.[66] In fact, resolution of lactic acidosis appears to be a surrogate marker of response to anti-lymphoma therapy.[4] It is noteworthy that the lactic acidosis is being generated here by the tumor microenvironment, but the disappearance of the cancer cells did not reverse the corrupted microenvironment, since the patient continued to have an elevated LDH serum level. Initially, the clinicians were concerned about residual disease with the persistently elevated blood LDH level, but without evidence of disease, the elevation is likely related to the resistance of a metabolically transformed glycolytic stroma to revert back to normal. This may place the patient at risk for recurrent disease or secondary malignancies.

Lactic Acidosis Is Due to the Lymphoma—Excluding Other Possible Causes

The patient had multiple potential causes for his lactic acidosis. He had a lymphoma, cirrhosis, mild renal dysfunction, and was taking metformin. However, we believe that the major culprit of his lactic acidosis was the lymphoma itself. In favor of the acidosis being mainly due to the lymphoma is the fact that it corrected after achieving control of the lymphoma. It also is extremely rare for patients with cirrhosis and severe hepatic dysfunction to develop lactic acidosis.[11,67,68]

The patient described here also had mild renal insufficiency, and the kidneys also metabolize and clear lactic acid.[69,70] Renal lactic acid can enter either the biosynthetic pathway to generate glucose or the oxidative phosphorylation pathway to generate CO_2 and water.[71] Despite its contribution to lactic acid clearance, the kidneys only clear 10% of total lactic acid, even in cases of lactic acid load.[72–74] Hence, it is unlikely that kidney dysfunction is the root cause of this patient's lactic acidosis.

Finally, this patient had diabetes mellitus and was being treated with metformin. However, metformin treatment has been only very rarely reported to cause lactic acidosis, with approximately 4 cases per 100,000 patient-years.[75,76] In our patient, metformin was held due to the lactic acidosis. Once the acidosis resolved, metformin was resumed without recurrence of the acidosis, which makes metformin an unlikely culprit. In summary, our patient's lactic acidosis was most likely due to increased lactic acid production by the tumor microenvironment.

Lactic Acidosis as a Paraneoplastic Syndrome

Paraneoplastic syndromes are manifestations of cancer not caused directly by cancer cells, but due to secretion of cytokines, peptides, hormones, or cross-reactivity between malignant and normal cells.[77] They have been of great interest to clinicians because of the difficulty in diagnosing them. In particular, paraneoplastic syndromes have a broad clinical spectrum, can potentially affect any organ or system, may precede or follow the diagnosis of the cancer, and do not parallel the clinical course of the neoplasm.

We now recognize the important systemic effects of cancer not attributable to direct tumor invasion or compression and the high frequency of paraneoplastic phenomena such as malaise, fevers, cachexia, and metabolic derangements.[78] For example, memory loss, confusion, and personality changes with disinhibition can arise as a result of limbic encephalitis without cancer invasion in a wide variety of cancer types, including lymphomas, and the diagnosis can be missed because, in some cases, the cancers are otherwise non-aggressive.[79–82] Not surprisingly, FDG-PET scans are a promising imaging technique for paraneoplastic syndromes, both to diagnose the tumor and the paraneoplastic encephalitis.[83,84] This is the first report to our knowledge of lactic acidosis as a paraneoplastic phenomenon, as lactic acid is being generated by stromal cells.

In summary, it is well accepted that energy metabolism reprogramming and transformation of the tumor microenvironment are hallmarks of cancer.[85] The novel finding in this patient is that lactic acidosis is a paraneoplastic phenomenon with lymphoma cells reprogramming stromal cells, as seen by high expression of PKM1, PKM2, LDHB, and MCT4 in stromal cells. This high extracellular lactic acid level supports mitochondrial OXPHOS metabolism in lymphoma cells (see Figure 8). Stromal transformation with lactic acid production and export persists after achieving a complete remission (CR) of the lymphoma as shown by the persistently elevated LDH and fibrosis. We can conclude that this patient's lymphoma is an example of metabolic coupling between different tumor cell compartments. Metabolic coupling within tumors should be investigated to determine if it can be exploited diagnostically, prognostically, and therapeutically.

We propose the following questions: (1) Are there any effective strategies to decrease his risk of recurrence or secondary malignancies? (2) How should this patient be monitored to detect recurrence or secondary malignancies?

MEDICAL ONCOLOGISTS' OPINION

Different strategies have been evaluated to decrease the risk of lymphoma recurrence. Maintenance rituximab is effective in low-grade lymphomas to improve progression-free-survival (PFS).[86] However, there are conflicting data on the value of maintenance rituximab in DLBCL. Some prospective studies have not found any differences in PFS or OS,[87] while others demonstrated that maintenance rituximab after R-CHOP improved PFS, although OS only improved for patients with IPI ≥ 3.[88] It is not routine practice to administer rituximab maintenance in DLBCL.[89]

While this patient was not eligible for an autologous peripheral blood stem cell (PBSC) transplant because of severe liver disease the use of autologous stem cell transplant as part of upfront therapy for patients with high-risk features in first CR has been the subject of multiple randomized trials. Unfortunately, the definition of "high risk" varies between the various trials and the fact that some trials enrolled partial responders as well and the inclusion of other types of lymphoma beside DLBCL. Furthermore, the standard- and high-dose therapy arms varied significantly between studies and sometimes up to 25% of study subjects could not complete their study treatment. One meta-analysis found that there was no benefit in terms of event-free survival or OS although there was a suggestion of a benefit in those with poor-risk features (IPI > 1).[90] A more recent abstract summarizing the results of Southwest Oncology Group (SWOG) trial S9704 comparing eight cycles of R-CHOP to six cycles of R-CHOP followed by autologous PBSC transplant (PBSCT) in patients with high-intermediate or high IPI found improved PFS in the early autologous PBSCT group.[91] Thus for patients with high-risk DLBCL in first CR there may be a benefit to early autologous PBSCT and such patients should be referred to a transplant center early on in treatment and consider enrollment in a clinical trial.

This patient's lymphoma was found to have metabolic and autophagy compartmentalization with increased OXPHOS metabolism in the cancer cell compartment and increased autophagy in the lymphoma microenvironment. Use of modulators of OXPHOS metabolism and autophagy such as chloroquine (CQ), hydroxychloroquine (HCQ), temsirolimus, and metformin may be novel treatments for cancers with this type of compartmentalization. CQ or HCQ are US Food and Drug Administration (FDA)-approved drugs that inhibit autophagy and that can attenuate tumor growth in cancer cell lines and in mouse models.[92] Multiple ongoing clinical trials are using CQ or HCQ for anticancer therapy, either alone, or in combination with other anticancer drugs.[93,94] Metformin inhibits mitochondrial OXPHOS metabolism by blocking mitochondrial electron transport and it is also an activa-

tor of adenosine monophosphate (AMP)-activated protein kinase (AMPK). AMPK activation, in turn inhibits protein synthesis and gluconeogenesis during cellular stress, induces cell cycle arrest, and apoptosis and reduces growth factor signaling. Also, AMPK activation induces autophagy by inhibiting the mammalian target of rapamycin (mTOR). Inhibitors of mTOR have been evaluated in phase II studies in relapsed/refractory mantle cell lymphoma.[95] A phase I study with the combination of metformin and the mTOR inhibitor temsirolimus for patients with lymphoma is currently ongoing.[96] In summary, treatments that target autophagy and mitochondrial metabolism may be effective for cancers with metabolic and autophagy compartmentalization.

This patient is at high risk of recurrence with a high IPI score, lactic acidosis, persistent LDH elevation, and multiple cancer cell clones. Hence, careful monitoring for recurrence should be performed. Evaluation of minimal residual disease (MRD) for recurrent or relapsed lymphoma is being investigated in non-Hodgkin lymphoma (NHL), especially in mantle cell lymphoma and follicular lymphoma.[97,98] In DLBCL, MRD can be detected in up to 70% of cases identifying the clonal B cells with a specific IgH variable region (VH) sequence.[99] In this patient, in addition to studying rearrangements in the IgH variable region, LDH and lactate levels should be monitored to assess for recurrent disease. In this case, the elevated lactate levels would be generated by the reactive stromal cells, which support the aggressive growth of anabolic lymphoma cells. This elevated lactate is generated via the enzyme LDH and serum LDH levels are a prognostic biomarker in DLBCLs and a component of the IPI score for aggressive NHL. This patient shows us that the prognostic biomarker LDH may not be a cancer cell biomarker, but instead a stromal cell biomarker. It is possible that serial measurements of LDH may be useful to detect MRD and quantitate how much reactive or transformed stroma is present.

FDG-PET is an important radiologic tool for staging in DLBCL. Its value as an early predictor of refractory and relapsed disease has been questioned. Prospective studies have shown high false positive rates in DLBCL and failed to demonstrate recurrent lymphoma in tissue biopsies obtained from positive FDG-PET scans after one to three cycles of treatment.[100,101] In a patient like the one presented, a positive FDG-PET scan would most likely be related to the tumor stroma cells that have very high glucose metabolism, rather than due to the cancer cells.[19] Some experiments with L-3- ^{11}C-lactate as a PET tracer for lactate metabolism have been evaluated in animal models,[102] and this type of PET tracer could be useful in cancers with the type of metabolic compartmentalization and uptake of lactate by the cancer cells described in this patient.

In addition to a high risk of lymphoma recurrence, this patient is also at risk for secondary malignancies. These secondary cancers are well described after treatment of DLBCL and are potentially related to chemotherapy and radiotherapy, underlying immune dysfunction, immunodeficiency, viral infections, tobacco, and genetic susceptibility.[103] The most common secondary malignancies are acute myeloid leukemia and lung cancer. In a retrospective follow-up study, the cumulative incidences at 5 and 10 years of secondary myelodysplasia/acute leukemia (sMDS/AL) were 3.09% and 4.52%, respectively, and that of solid tumors were 2.54% and 6.79% at a median follow-up of 7 years.[104] Some factors, such as age, concomitant radiotherapy, and the addition of rituximab may have contributed to the development of secondary tumors. After autologous transplant, factors related to secondary malignancies were advanced age, post-transplant radiation, and previous rituximab.[105] Unfortunately, there is no effective strategy to prevent secondary malignancies in lymphoma survivors. The focus in clinical practice is surveillance to detect these secondary malignancies, although it is unknown if early detection improves outcomes. There is a general lack of clinical trials focused on screening and preventative practices for NHL survivors. In pediatric Hodgkin lymphoma, where the incidence of secondary malignancies is higher compared to the general population, there are prospective studies that support effective strategies for secondary prevention.[106] In general, recommendations provided by the National Comprehensive Cancer Network (NCCN) guidelines are avoidance of exposure to radiation, periodic skin self-examination, and breast and genital/testicular exams. Patients should be counseled to avoid high-risk behaviors that would increase the risk of cancer, including smoking, passive tobacco exposure, and excessive unprotected skin exposure to UV light. Patients should be seen yearly and the evaluation, which would include symptom review for secondary malignancy. And finally, patients should follow the general population recommendations for cancer screening such as screening for colon cancer with colonoscopy and PAP smears.

Metformin use due to its inhibitory effect on OXPHOS metabolism to prevent secondary malignancies should be assessed. Studies evaluating metformin in primary prevention in diabetic patients have shown a decreased incidence of cancer.[107]

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MEDICAL ONCOLOGISTS' OPINION

The patient described in this case report has a pathologic diagnosis of DLBCL, stage IVBE, but with no visual presence of lymphoma involving the bone marrow but suspected due to a positive BCL6 rearrangement detected by FISH. The bulk of the disease was located in the abdominal cavity with prominent lymphadenopathy, malignant ascites, innumerable diffuse splenic lesions, and peritoneal implants. There were other sites of disease, as well including axillary, mediastinal, and pericardiophrenic lymphadenopathy. The pathologic diagnosis was obtained via sampling of the ascitic fluid, excisional lymph node biopsy from the right axillary adenopathy, and a bone marrow biopsy. The ascitic fluid revealed a population of lymphocytes that was positive for CD19 and CD20, negative for CD5 and CD10, and lambda light chain–restricted, which confirmed that this was a malignancy arising from mature B lymphocytes. The lymph node biopsy stained positive for CD20, BCL 6, and lambda light chain restriction, which was similar to the phenotype noted in the ascitic fluid. Additionally, the Ki-67 stain on the lymph node biopsy was positive in 95% of the cells, indicating a very high rate of proliferation. As noted, the bone marrow biopsy did not display visible disease and the cytogenetics were therefore likely normal male karyotype. Thus, the malignancy in the abdominal cavity and the axillary lymphadenopathy are the same and the bone marrow biopsy is highly suggestive of involvement as well. The R-IPI was 4, which is associated with an estimated OS of 55% at 5 years.

Therapy with R-CHOP was initiated. However, one could argue that due to the high Ki-67 staining that a more aggressive regimen such as hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with cytarabine and methotrexate) would result in a better success rate. A key finding in this case is the absence of the c-MYC translocation which, when coupled with a BCL6 mutation, is defined as a "double hit" lymphoma and is associated with a poor survival.[108–110] However, one wonders if the c-MYC translocation would have been found in the peritoneal implants. Once such a patient has achieved a remission they should be considered for high-dose chemotherapy followed by autologous stem cell rescue. I believe this should be considered only if the patient has had a partial remission and a subsequent bone marrow biopsy does not reveal any visible lymphoma involvement. The role of high-dose chemotherapy and autologous stem cell rescue upon first remission in patients with a high IPI score was supported by the prospective, randomized LNH87-2 trial.[111] This study revealed that patients with a high IPI who had consolidation with high-dose chemotherapy followed by autologous stem cell rescue had a superior 8-year survival rate as compared to those who received induction chemotherapy alone. The SWOG S9704 study confirmed the advantages of an autologous transplant in the rituximab era.[91] Unfortunately, the patient presented in this case has a history of hepatitis C with advanced liver disease, which would be a contraindication to high-dose chemotherapy and autologous stem cell rescue. This patient received induction chemotherapy with the R-CHOP regimen, which may be appropriate given his comorbidities.

Following the completion of six cycles of chemotherapy with the R-CHOP regimen, the patient achieved a PET-negative remission. There is no identified role of maintenance therapy with any chemotherapeutic or immunobiologic agents such as rituximab. This patients' lymphoma should be monitored every 3–4 months with a physical examination, laboratory studies including an LDH, and a PET/CT. However, the patient also has hepatitis C and advanced liver disease, which needs attention with serum alpha-fetoprotein and an ultrasound performed at least every 6 months as recommended by the American Association for the Study of Liver Diseases.[112]

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DISCUSSION AND CONCLUSION

The patient developed high fevers and pancytopenia 7 months after completing treatment for his DLBCL. He underwent bone marrow biopsy, which revealed acute myeloid leukemia with a translocation between chromosomes 9 and 11 and mixed lineage leukemia (*MLL*) gene rearrangement. Acute myeloid leukemia with *MLL* gene rearrangement is most commonly observed after chemotherapy treatment with anthracyclines. He was treated with azacytidine but died from a pulmonary hemorrhage due to disseminated intravascular coagulation within 3 months of the diagnosis.

We want to thank the Medical Oncology experts for their perspectives on surveillance and maintenance options. Unfortunately, the current standard of care for patients is active surveillance for recurrence and secondary malignancies without maintenance therapy. We hypothesize that a metabolic reprogramming strategy may have prevented the development of acute myeloid leukemia. This hypothesis is based on the detailed metabolic characterization of his tumor with persistent elevation in LDH. It is very likely that the stroma continued to be reactive and tumorigenic. The field of metabolic interactions between cancer cells and the mic-

roenvironment is an emerging field. Preclinical studies show that FDA-approved drugs such as metformin, CQ, and sirolimus can modulate cancer–stromal metabolism. Clinical trials evaluating metabolic reprogramming need to be implemented.

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Note Added in Proof:

In accordance with our current findings in B-cell lymphoma, we have recently shown that Ki-67-positivity (a well-established marker of cell proliferation) directly correlates with increased expression of markers of mitochondrial mass (TOMM20), in aggressive head and neck cancers.

Thus, rapid cell proliferation in tumor cells most likely reflects an amplification of mitochondrial power in cancer cells, fueled by high-energy stromal-derived metabolites, such as L-lactate.

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