CLINICAL LAB INVESTIGATIONS: CASE STUDIES FOR THE LABORATORY PROFESSIONAL

CASE SET #21

A Hematology Case:

Follicular Lymphoma

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Clinical Laboratory Investigations

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LEARNING OBJECTIVES

Upon completion of reading the case, the learner will be able to:

1. Identify the symptoms and laboratory results of a patient with Follicular Lymphoma.

2. Compare normal and abnormal B-cell maturation, and how genetic translocations can lead to cancer.

3. Discuss how follicular cells are able to control the microenvironment.

4. Describe the grading progression and treatment involved in Follicular Lymphoma.

Follicular Lymphoma

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Case Presentation 2014:

A 73-year-old male presented to his primary care physician with left groin pain. The patient had a history of inguinal hernia, dislocated hip, fractured hip socket, hypercholesterolemia, coronary artery disease, hyperglycemia, thrombocytopenia, and osteopenia. He weighed 190 pounds and reported smoking 1-3 cigarettes per day. The patient complained of a sudden burning pain in his left groin that appeared after he stood for a long period of time. Ibuprofen relieved the pain within 20 minutes. The spine and hip showed full range of motion, eliminating compression as the cause of his pain. Lack of numbness ruled out entrapment of the lateral femoral cutaneous nerve (LFCN), a syndrome known as meralgia paraesthetica or Bernhardt-Roth Syndrome.[1] The left inguinal lymph node was enlarged, localized, and hard, indicative of lymphadenitis, malignancy, or reactivity. The patient's wife was concerned he was sleeping too much, however the patient stated he had not been feeling tired. The physician ordered a comprehensive metabolic panel (CMP), complete blood count (CBC) with differential, ultrasound, chest X-ray, and excisional biopsy of the lymph node.

The patient's initial laboratory test values were consistent with his previous results and history. His serum glucose (123 mg/dL with a reference range of 74-100 mg/dL) and lactate dehydrogenase (LDH) (280 U/L with a reference range of 87-241

U/L) remained elevated from previous testing. The patient had been previously diagnosed with hyperglycemia. The CBC showed a decreased platelet count, consistent with his history of thrombocytopenia. The radiological picture of his chest X-ray remained unchanged. An ultrasound revealed a markedly enlarged 4 cm lymph node (average size is 0.5-2.0 cm), indicative of lymphadenopathy (LAD) of his left inguinal lymph node.

One week later, the lymph node showed considerable shrinkage; however, the patient still experienced intermittent burning in his left groin. Upon palpitation, the left inguinal lymph node appeared pea-sized and firm, without tenderness or warmth. Also noted on the exam was slight LAD of the lymph nodes making up the right inguinal chain. This finding showed enlargement on both sides of the patient's groin, a change from one week prior. The differential diagnosis, provided by the attending physician, was lymphadenitis, malignancy, or reactivity causing lymph node enlargement. Reactivity associated with a viral infection such as an early precursor to shingles was a possibility. After considering the options presented to him, the patient declined a lymph node biopsy and requested a trial course of the antibiotic Cefadroxil (Duricef) even though a leg infection was not indicated by his laboratory results. The physician agreed with the plan and recommended proceeding with the biopsy if symptoms did not resolve in a week or two.

Case Presentation 2015:

One year later, the same patient presented to his physician with a cough, abdominal pain, loss of appetite, polydipsia, and polyuria. He weighed 172 pounds and

rated his pain a 7 on a scale of 0 - 10. His lungs were clear and did not present signs of distress. Cold symptoms, dyspnea, wheezing, chest pain, sore throat, fever, and chills were not present; however, the patient stated he coughed to the point of gagging or vomiting. He took Delsym, a cough suppressant, without relief. The patient reported cutting back on smoking in the last 3 months. His 18 pound weight loss was attributed to his unintentional decrease in appetite over the past 2-3 months. Left axillary and inguinal lymph nodes showed non-tender LAD. Axillary LAD was not present in 2014. The patient complained of experiencing night sweats over the previous 3 weeks.

The physician ordered X-ray computer tomography (CT) of the chest with contrast due to his coughing and axillary LAD, and a CT of the pelvis due to his inguinal LAD. The thorax CT with contrast revealed an increase in the number of mediastinal and axillary lymph nodes. Mild degenerative changes in the patient's thoracic spine were expected. Hepatic cysts with spleen enlargement, intra-abdominal, intra-pelvic, and inguinal lymphadenopathy were present on the pelvic CT scan. Other abnormalities were noted but remained unchanged from historical CT scans.

Based upon these findings and the patient's symptoms, the physician ordered a CBC with a differential, CMP, and urinalysis without microbiology culture. Notable results are shown in Tables I, II, III and Figure I.

Table I: **Urinalysis Results, 2015**

Test	Results	Reference Range	Abnormal Results
Color	Yellow	Clear - Straw	
Specific Gravity	1.015	1.015 - 1.025	
pH	5.0	5 - 9	
Leukocytes	0	Negative	
Nitrite	0	Negative	
Protein	0	Negative	
Urine Glucose	0	Negative	
Ketones	1	Negative	*Elevated*
Urobilinogen	4	Negative	*Elevated*
Bilirubin	1	Negative	
Blood	0	Negative	

Table II: **Comprehensive Metabolic Panel (CMP) Notable Results, 2015**

Test	Results	Reference Range	Abnormal Results
Lactate Dehydrogenase (LDH)	448	87-241 U/L	*Elevated*
Glucose	93	74-100 mg/dL	
Albumin	3.2	3.4 – 5.0 g/dL	*Low*
Alkaline Phosphatase (ALP)	144	45 – 117 U/L	*Elevated*
Aspartate Aminotransferase (AST)	56	5 – 37 U/L	*Elevated*

Alanine Aminotransferase (ALT)	39	7 – 65 U/L	

Table III: Complete Blood Count (CBC) with Differential, 2015

Test	Results	Reference Range	Abnormal Results
White Blood Cells (WBC)	25.02	4.23 – 9.07 x 10^3/□L	*Elevated*
Red Blood Cells (RBC)	4.03	4.63 – 6.08 x 10^3/□L	*Low*
Hemoglobin (HGB)	12.4	13.7 – 17.5 g/dL	*Low*
Hematocrit (HCT)	38.5	40.1 – 51.0 %	*Low*
MCV	95.5	80.0 – 96.0 fl	
MCH	30.8	25.7 – 32.2 pg	
MCHC	32.2	32.3 – 36.5 g/dl	*Low*
RDW	16.0	11.3 – 14.4 %	*Elevated*
Platelet Count	126	150 - 400 x 10^3/□L	*Low*
Neutrophils	12.0	36.0 - 74.0 %	*Low*
Lymphocytes	87.0	27.0 - 43.0 %	*Elevated*
Monocytes	1.0	3.0 - 11.0 %	*Low*
Nucleated RBC (NRBC)	1.0	0/100 WBC	*Elevated*
Neutrophil Number	3.00	1.40 – 7.70 X 10^3/□L	
Lymphocyte Number	21.77	1.10 – 4.00 X 10^3/□L	*Elevated*
Monocyte Number	0.25	0.10 – 1.10 X 10^3/□L	
WBC Abnormalities	Many reactive lymphocytes and polychromasia noted		

Platelet Estimate	Decreased		

<u>Figure I:</u> Abnormal cells found on patient's 2015 peripheral blood smear.

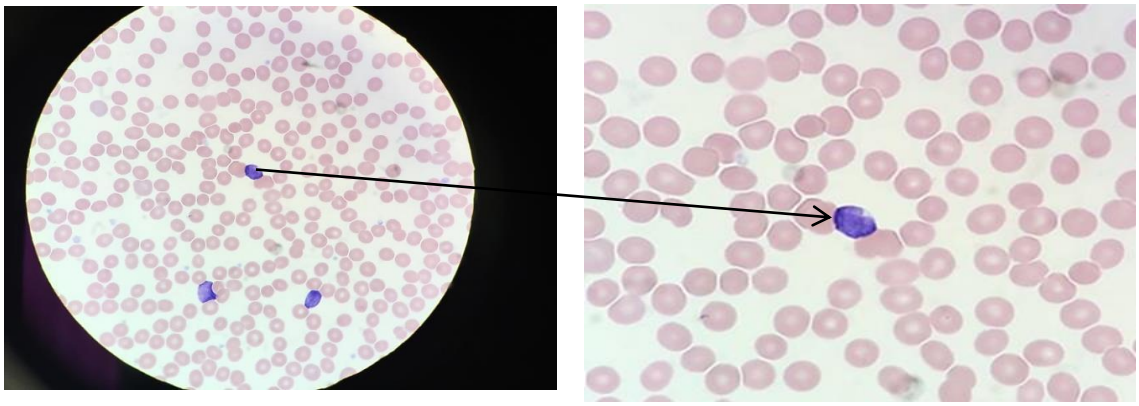

Photo taken by author

The patient's CBC with differential showed thrombocytopenia consistent with his history. Low RBC, HCT, and HGB results suggested anemia. Urobilinogen is a byproduct of bilirubin breakdown associated with hepatocellular and hemolytic conditions.[2] The patient's elevated urine urobilinogen, presence of NRBC, polychromasia, and probable anemia indicated mild RBC hemolysis, secondary to his spleen enlargement.

The patient's hepatic cysts caused hepatic impairment, which was seen in his liver enzyme results and albumin production. Low albumin indicated production impairment and a high ALP liver enzyme indicated disruption of bile production.[3] His low AST to ALT ratio confirmed the non-alcoholic nature of his liver disease.[4] Mild impairment of liver enzyme synthesis was evident due to his hepatic cysts, but secondary to his main diagnosis.

The patient's LDH jumped from 280 U/L in 2014 to 448 U/L in 2015. Elevated LDH indicated liver disease, hemolytic anemia, or abnormal tissue formation, such as cancer. The final diagnosis of this patient correlated with his elevated LDH.[5] The patient's decreased appetite with weight loss led his body to compensate for lack of nutrition by breaking down body fat creating a by-product of elevated urine ketones.[6]

An elevated WBC count with lymphocyte elevation signified leukocytosis and lymphocytosis. A depletion in neutrophils and monocytes supported the predominance of lymphocytes. His peripheral lymphocytes (Figure I) were reactive with a notched nucleus, suggesting malignancy. Upon pathologist's review, flow cytometry was ordered (Table IV).

Table IV: Peripheral Blood Flow Cytometry Results, 2015

Component	Result	Control Patient
Leuk/Lymph %CD45	80% Lymphocytes 17% Myeloid 3% Monocytes	83% Myeloid 14% Lymphocytes 3% Monocytes
Leuk/Lymph %CD2	11%	81%
Leuk/Lymph %CD3	12%	70%
Leuk/Lymph %CD4	7%	47%
Leuk/Lymph %CD5	10%	68%
Leuk/Lymph %CD7	13%	73%
Leuk/Lymph %CD8	4%	24%
Leuk/Lymph %CD16	3%	18%
Leuk/Lymph %CD10	**74%**	5%
Leuk/Lymph %CD19	**84%**	8%

Leuk/Lymph %CD20	**76%**	5%
Leuk/Lymph %Kappa	81%	Variable
Leuk/Lymph %Lambda	<1%	Variable
Leuk/Lymph %CD34	<1%	2%
Leuk/Lymph %CD13	1%	2%

Of the CD45 positive WBC, 80% were lymphocytes, 17% were myeloid and 3% were monocytes. Of the lymphocytes, 84% were B-cells, 12% were T-cells, and 2% were Natural Killer cells, indicative of predominating B-cells. The B-cells expressed kappa light chains CD10, CD19, and CD20. The T-cells were phenotypically normal and had a CD4:CD8 ratio of 1.8 (with an age specific reference range of 0.80 to 6.17). Myeloid cells and monocytes expressed normal patterns. Due to the increase in the patient's peripheral WBC count, absolute lymphocyte count, predominating B-cells, and B-cell markers, the patient was diagnosed with a B-cell lymphoproliferative disorder. Markers CD10, CD19, and CD20 were consistent with Non-Hodgkin Lymphoma (NHL) of germinal center cell origin.

A renowned cancer institute reviewed the patient's case and concluded that flow cytometry results were consistent with Non-Hodgkin's Lymphoma, but suggested an inguinal node biopsy should be performed for further classification. They recommended treatment with a systemic chemotherapy agent with targeted therapy including rituximab. However, sub-typing the NHL would provide a definitive therapy treatment.

Microscopy with immunoperoxidase stain of the inguinal node biopsy showed BCL-2, Ki-67, CD20, CD3, and CD10 positive monoclonal kappa B-cell population consistent with Follicular Lymphoma (FL), a subtype of NHL. An increase in large cells indicated progression to Grade 3 with a high proliferation index suggestive for an aggressive treatment, such as rituximab**.** Marker Ki-67 positive indicated proliferation, a commonality among cancers. Positive BCL-2 protein is indicative of a genetic translocation (t(14;18)(q32;q21)) causing FL.[7]

Flow cytometry performed on the left groin lymph node biopsy revealed that all CD45 positive leukocytes were lymphocytes with 70% B-cells and 27% T-cells. The T-cells were phenotypically normal but showed a CD4:CD8 ratio of 8.0 (with an age specific reference range of 0.80 to 6.17). Of the B-cells, CD10, CD19, and CD20 were expressed. CD5 was not expressed, consistent with most FL cases. These results were consistent with the peripheral blood flow cytometry and verified the patient's diagnosis of FL.

Discussion:

This case study depicts the progression and complexity of diagnosis of FL. Further classification leads to specific and accurate treatment and prognosis. In the 2015 presentation of this patient, it was evident he had LAD and lymphocytosis consistent with lymphoma. The peripheral blood flow cytometry classified it as Non-Hodgkin's Lymphoma. The flow cytometry showed the lymphocytes were predominantly of B-cell origin, rather than of T-cell origin. To further differentiate, flow cytometry of the lymph node biopsy indicated B-cell antigen markers consistent with

Follicular Lymphoma. The markers and cell size enabled grading of the FL. This specific classification allowed for precise and accurate treatment tailored to Grade 3 Follicular Lymphoma. Figure II shows the breakdown of this patient's lymphoma classification.

<u>Figure II:</u> Follicular Lymphoma classification

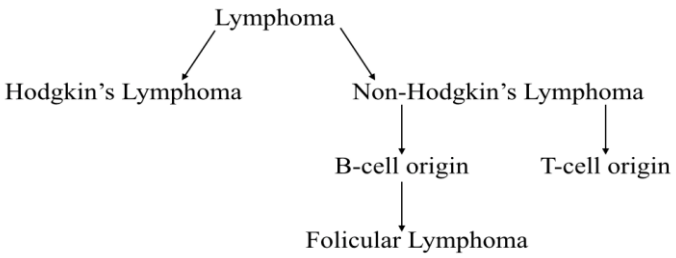

Figure based on: The 2008 WHO classification of lymphoid neoplasms and beyond[8]

Follicular lymphoma (FL) is the second most common type of NHL, accounting for 35% of cases. Lymph node enlargement is a feature of classic FL presentation, with 20% of cases experiencing fever, night sweats, and unintentional weight loss. Laboratory results are usually inconclusive and tend to exhibit secondary issues to FL. In 25% of cases, an elevated LDH or cytopenia is seen.[7] This patient presented with weight loss, night sweats, increased LDH, and decreased RBC and platelet count consistent with FL symptoms.

In FL, a malignant transformation causes proliferation of immature B-cells in lymphoid organs. In 85% of FL cases, t(14;18)(q32;q21) is the genetic driving factor.[7]

However, in 50% of the healthy adult population only 0.03% of this population will develop FL. Therefore, t(14;19)(q32;q21) is not used as a predictive biomarker but rather supports diagnosis of FL. This translocation causes B-cell leukemia/lymphoma 2 (BCL-2), oncogene on chromosome 18, to translocate with silent immunoglobulin heavy locus (IGH) allele on chromosome 14. This causes IGH enhancers to operate BCL-2 gene creating a non-apoptotic BCL-2 protein. An immunoperoxidase stain of the patient's inguinal node biopsy showed BCL-2 expression supporting FL diagnosis.[9]

In t(14;19)(q32;q21) FL, an increase in BCL-2 expression from the translocation gives the immature cells anti-apoptotic properties.[10] During B-cell differentiation in the bone marrow, V(D)J recombination diversifies immunoglobulin heavy and light chains to create unique antigen receptor genes. Normally, if a non-functional premature B-cell is formed, then the cell undergoes apoptosis. If the V(D)J recombination forms a functional premature B-cell, then the naïve B cell migrates to the germinal center of a lymphoid organ. The germinal center is an area for B lymphocytes to proliferate and undergo clonal expansion and diversification. In the germinal center, the naïve B-cell encounters antigens, CD4+ T-cells, and APC signals prompting further maturation.[11] A mistake in the V(D)J recombination leads to a non-functional immature B-cell, however, apoptosis is prevented due to the increase in BCL-2 expression.[10] This is mapped out in Figure III.

<u>Figure III:</u> Functional versus Non-Functional Pre-B-Cell V(D)J Recombination

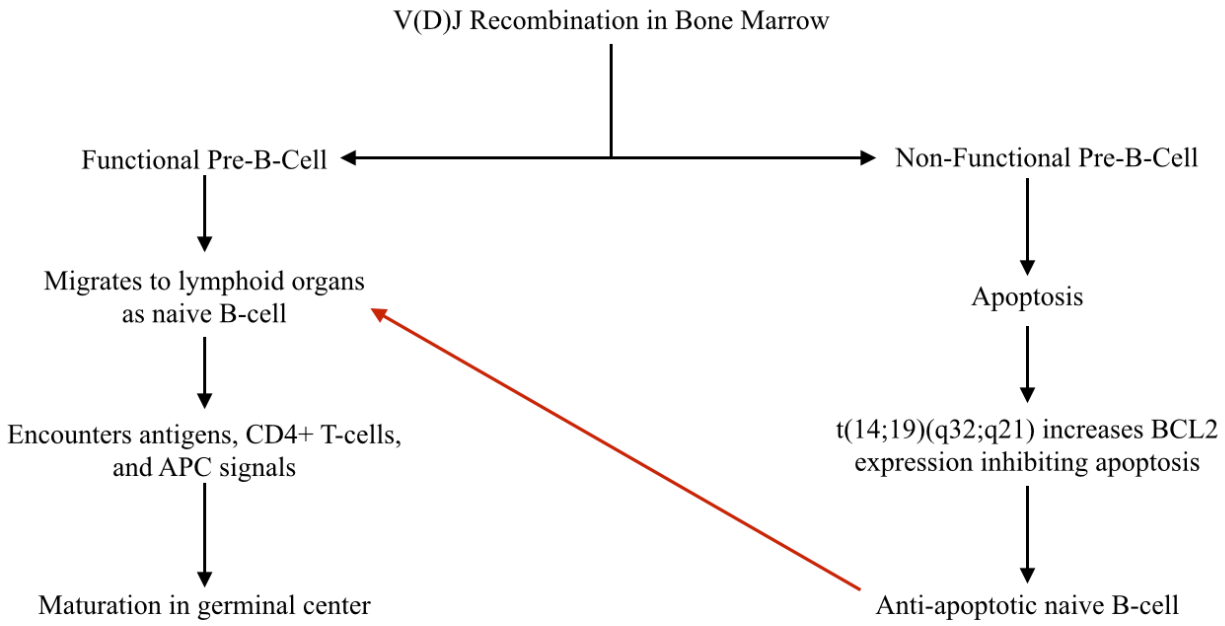

Figure based on The Biology of the Germinal Center[10]

The naïve B-cell is in the germinal center of a lymphoid organ where it receives signals to further differentiate into a centroblast.[11] Somatic hypermutation (SMH), nucleotide substitutions, deletions, and duplications are randomly introduced into the Ig variable (IgV) regions of centroblasts to create diverse antigen specificity.[12] BCL-6, a master regulator and transcription repressor seen on centroblasts, allows rapid proliferation and DNA modification by SMH to occur in centroblasts.[10,11] Lack of high affinity antibodies, mutations in the IGV regions, or auto-reactivity cause centroblasts to undergo apoptosis. In FL, BCL-2 expression increases inhibiting apoptosis, therefore, the cell continues to differentiate into a centrocyte even in the presence of a malformation.[10,11]

A centroblast further differentiates into a centrocyte, which expresses high affinity B-cell receptors (BCR) to receive CD40 signals. CD40 signals down-regulate BCL-6 allowing the centrocyte to differentiate into a memory or plasma B-cell.[11] This process is outlined in Figure IV.

Figure IV: Normal versus Anti-Apoptotic Naïve B-cell Maturation to a Memory or Plasma B-cell

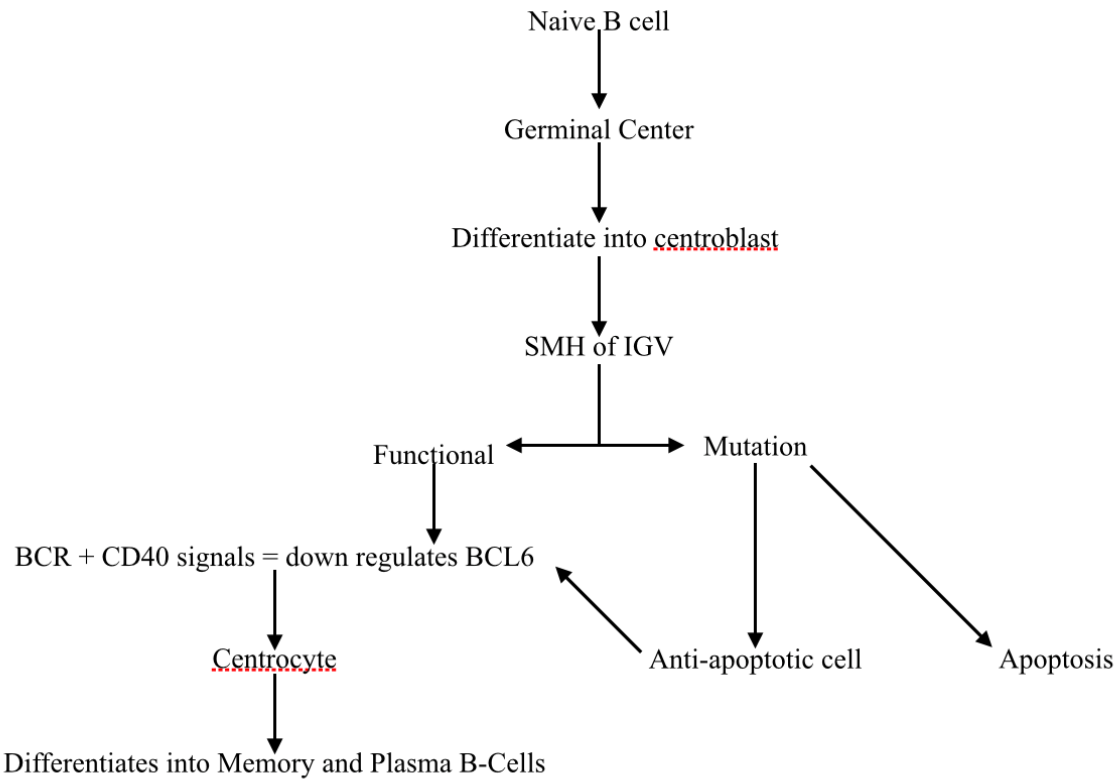

Figure based on Germinal centers and B-cell lymphomagenesis[11]

In FL, studies show an increase of CD4 positive T-cell and a decrease in CD8 positive T-cell (high CD4/CD8 ratio) lymph nodes. CD4 positive follicular helper T-cells support FL, and CD8 positive cytotoxic T-cells inhibit FL cells. This gives FL cells an

incentive to recruit CD4 positive cells and suppress CD8 positive cells. FL increases IL-4 receptor gene expression, which is secreted by CD4 positive T-cells to regulate proliferation, differentiation, and apoptosis of B-cells. The patient's first flow cytometry analysis showed a 1.8 CD4:CD8 ratio. His second, on the left groin lymph node, showed a CD4:CD8 ratio of 8.0. This indicates FL cells alter their immune microenvironment to enhance development.[13]

FL is divided into grades; 1, 2, 3A and 3B. In Grade 3A, centrocytes are visible and in Grade 3B, lymph nodes are completely centroblasts.[8] The lymphoma cells in the patient's peripheral blood smear had a notched nucleus indicative of centrocytes. The chromatin of these premature B-cells is loosely clumped with less intense staining, consistent with Grade 3.[14] The patient had an increase in large cells indicating progression into centroblasts; therefore, an aggressive treatment was recommended.

In this case, a renowned cancer institute recommended rituximab treatment, an immunotherapy with anti-CD20 antibodies, targeting CD20 cells. Rituximab is an effective treatment for FL. It is often combined with a chemotherapy agent, such as bendamustine. This combination of bendamustine with rituximab (BR) is preferable treatment for aggressive late stage cancers, such as in this case study. BR is known to lower the chance of progression with low toxicity.[14]

Conclusion:

The patient's case study showed normal progression of Follicular Lymphoma; however, in most cases FL is caught earlier. Due to his choice of denying a lymph node biopsy in 2014, the cancer metastasized to several lymph nodes and lymphoid organs.

The patient's spleen enlargement with hemolysis and hepatic cysts with elevated liver enzymes suggested possible metastasis to the spleen and liver. The centrocytes in his peripheral blood smear indicated late grade of FL and the incurability of the progression. The patient's early CMP and CBC were inconclusive of a disease state; however his disease became apparent in the 2015 laboratory testing. The patient's results illustrate cancer's utilization of the immune system, via CD4 enhancement and CD8 suppression, to grow and develop. Unfortunately, this patient's disease was diagnosed too late for treatment to be effective, and he passed away due to complications associated with FL.

REFERENCES

1. Pearce, J. M. S. (2006). Meralgia paraesthetica (Bernhardt Roth syndrome). Journal of Neurology, Neurosurgery, and Psychiatry, 77(1), 84. http://doi.org/10.1136/jnnp.2005.07236.
2. Somerville, J. A., Maxted, W. C., & Pahira, J. J. (2006). Urinalysis: a comprehensive review. American Family Physician, 71(6), 1153-1162. http://www.aafp.org/afp/2005/0315/p1153.html.
3. Martin, L. J., Ogilvie, I., & Zieve, D., (2015). ALP- Blood test. U.S. National Library of Medicine.
4. https://www.nlm.nih.gov/medlineplus/ency/article/003470.htm.
5. Botros, M., & Sikaris, K. A. (2013). The De Ritis Ratio: The Test of Time. The Clinical Biochemist Reviews, 34(3), 117–130. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3866949/.
6. Gersten, T., Ogilvie, I., & Zieve, D. (2014). Lactate dehydrogenase test. U.S. National Library of Medicine. https://www.nlm.nih.gov/medlineplus/ency/article/003471.htm.
7. Black, B., Wisse, B., & Zieve, D. (2013). Ketones urine test. U.S. National Library of Medicine. https://www.nlm.nih.gov/medlineplus/ency/article/003585.htm.
8. Aster, J. C., & Freedman, A. S. (2015). Clinical manifestations, pathologic features, diagnosis, and prognosis of follicular lymphoma. Available from http://www.uptodate.com/contents/clinical-manifestations-pathologic-features-diagnosis-and-prognosis-of-follicular lymphoma?source=search_result&search=follicular+lymphoma&selectedTitle=1%7E124.
9. Campo E., Swerdlow S., Harris N., Pileri S., Stein H., & Jaffe E. (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood Cancer Journal. 177(19):5019-5032. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3109529/.
10. Mamessier, E., Broussais-Guillaumot, F., Chetaille, B., Bouabdallah, R., Xerri, L., Jaffe, E. S., & Nadel, B. (2014). Nature and importance of follicular lymphoma precursors. Haematologica, 99(5), 802–810. http://doi.org/10.3324/haematol.2013.085548.
11. Natkunam Y. (2007) The Biology of the Germinal Center. ASH Education Book, 2007(1): 210-15. http://asheducationbook.hematologylibrary.org/content/2007/1/210.full.
12. Basso K. & Dalla-Favera R. (2015). Germinal centres and B-cell lymphomagenesis. Nature Reviews Immunology, 15: 172-184. http://www.nature.com/nri/journal/v15/n3/full/nri3814.html.
13. Honjo, T. & Kinoshita, K. (2001). Linking class-switch recombination with somatic hypermutation. Nature Reviews Molecular Cell Biology 2, 493-503.
14. Whalin B., Sander B., Christensson B., Ostenstad B., Holte H., Brown P., Sundstrom C. & Kimby E. (2012). Entourage: the immune microenvironment following follicular lymphoma. Blood Cancer Journal. 2(1):e52. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3270257/.

15. Freedman, A. S. & Friedberg, J. W. (2015). Initial treatment of advanced stage (III/IV) follicular lymphoma. Available from http://www.uptodate.com/contents/initial-treatment-of-advanced-stage-iii-iv-follicular-lymphoma?source=search_result&search=initial+treatment+of+advanced+stage+follicular+lymphoma&selectedTitle=1%7E150.