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Evaluation of Positive B- and T-Cell Gene Rearrangement Studies in Patients With Negative Morphology, Flow Cytometry, and Immunohistochemistry

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• ***Context.***—**The significance of positive immunoglobulin (IG) or T-cell receptor (*TCR*) gene rearrangement studies in the context of otherwise normal ancillary findings is unknown.**

Objective.—**To examine long-term hematologic outcomes of individuals with positive gene rearrangement studies with otherwise unremarkable blood or bone marrow studies in parallel.**

Design.—**Data from patients who underwent *IG* or *TCR* gene rearrangement testing at the authors' affiliated Veterans Affairs Hospital January 1, 2013 to July 6, 2018 were extracted from medical records. Date of testing, specimen source, and morphologic, flow cytometric, immunohistochemical, and cytogenetic characterization of the tissue source were recorded. Gene rearrangement results were categorized as test positive/phenotype positive (T+/P+), test positive/phenotype negative (T+/P–), test negative/phenotype negative (T–/P–), or test negative/phenotype positive (T–/P+) based on comparison to other studies and/or final diagnosis. Patient records were reviewed for subsequent diagnosis of hematologic malignancy for patients with positive gene rearrangements but no other evidence for a disease process.**

Results.—**A total of 136 patients with 203 gene rearrangement studies were analyzed. For TCR studies, there were 2 T+/P– and 1 T–/P+ results in 47 peripheral blood assays, as well as 7 T+/P– and 1 T–/P+ results in 54 bone marrow assays. Regarding IG studies, 3 T+/P– and 12 T–/P+ results in 99 bone marrow studies were identified. None of the 12 patients with T+/P– *TCR* or *IG* gene rearrangement studies later developed a lymphoproliferative disorder.**

Conclusions.—**Positive IG/TCR gene rearrangement studies in the context of otherwise negative bone marrow or peripheral blood findings are not predictive of lymphoproliferative disorders.**

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The diagnosis of a lymphoid malignancy requires the establishment of monoclonality of a lymphocyte population through morphologic assessment and laboratory testing, such as flow cytometry, immunohistochemistry, and cytogenetic analysis.[1] These methods can yield conflicting results, however, and in up to 15% of cases of suspected lymphoid malignancies, molecular assessment of immunoglobulin (*IG*) and/or T-cell receptor (*TCR*) gene rearrangements is necessary to confirm a diagnosis.[2] *IG*/*TCR* gene rearrangement testing is based on the principle due to V(D)J recombination in lymphocytes, the probability a population of B or T cells would share the same *IG* or *TCR* gene rearrangement is virtually zero. As such, the presence of a population of B or T cells with the same gene rearrangement pattern is highly supportive of lymphoid malignancy.[2]

Modern *IG*/*TCR* gene rearrangement analysis generally consists of manual or automated extraction of target DNA (eg, from peripheral blood, bone marrow aspirate, fresh or frozen tissue, or formalin-fixed, paraffin-embedded tissue) followed by multiplex polymerase chain reaction (PCR) and capillary gel electrophoresis.[3,4] Standardized primer sets and protocols are commercially available,[5] as are guidelines for a standardized approach to interpreting and reporting gene rearrangement results.[6] When IG/TCR clonality assessment is performed using these standardized reagents and protocols, detection of nearly all lymphoid malignancies is possible, including 99% of B-cell clones and 94% of T-cell clones.[7,8]

At times, results from morphologic assessment and other ancillary testing are benign or unrevealing despite the presence of an (apparent false) positive *IG* or *TCR* gene rearrangement result. The significance of positive rearrangement studies in the context of otherwise normal morphology and flow cytometry is unknown, and as such, we hypothesized gene rearrangement studies may be predictive of an emerging B- or T-cell clone in the absence of other abnormal laboratory tests, given the potentially higher sensitivity of molecular methods to detected abnormal

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Table 1. Demographics of the Study Population	
Total number of patients	136
Mean age at time of first testing	69.13
Sex	
Male	133 (98%)
Female	3 (2%)
Race	
White	111 (82%)
African American	19 (14%)
Native American	1 (<1%)
Native Hawaiian/Pacific Islander	2 (1%)
Declined to answer/data absent	3 (2%)
Ethnicity	
Hispanic	5 (4%)
Non-Hispanic	116 (85%)
Declined to answer/data absent	15 (11%)
Total number of gene rearrangement tests	203
Number of tests from peripheral blood	50
Number of tests from bone marrow	153

populations versus morphologic and even flow cytometric platforms.

METHODS

Data from all patients who underwent *IG* or *TCR* gene rearrangement testing at the authors' affiliated Veterans Affairs Hospital between January 1, 2013 and July 6, 2018 were extracted from the electronic medical record. Date of testing, specimen source, and morphologic, flow cytometric, immunohistochemical, and cytogenetic characterization of the tissue source were recorded from pathology reports. Positive gene rearrangement results were categorized as test positive/phenotype positive (T+/P+) if they corresponded with a pathologic diagnosis of a lymphoproliferative disorder, and test positive/phenotype negative (T+/P–) if they corresponded with normal or nonmalignant pathology results. Negative gene rearrangement results were categorized as test negative/phenotype negative (T–/P–) if they corresponded to normal or nonmalignant pathology results, or test negative/phenotype positive (T–/P+) if they corresponded to a pathologic diagnosis of a lymphoproliferative disorder. Last, patient records were reviewed for subsequent diagnosis of hematologic malignancy in patients with positive gene rearrangement results with negative ancillary testing. All gene rearrangement studies were performed according to BIOMED-2 protocol using fresh samples from peripheral blood (PB) and bone marrow (BM).[2]

RESULTS

A total of 136 patients, who had 203 gene rearrangement studies (50 PB and 153 BM), were analyzed. Demographic characteristics are found in Table 1. The patients were 133 men (98%) and 3 women (2%). Self-reported race was 111 patients white (82%), 19 African American (14%), 2 Native Hawaiian/Pacific Islander (1%), and 1 Native American (<1%), with 3 patients declining to answer (2%). Self-reported ethnicity was 116 non-Hispanic (85%) and 5 Hispanic (4%), with 15 patients declining to answer (11%). The mean age at time of first testing was 69 years.

Results from *IG* and *TCR* gene rearrangement studies from PB and BM are found in Table 2. In TCR studies, there were 2 T+/P– and 1 T–/P+ results in 47 PB assays using criteria established in methods, as well as 7 T+/P– and 1 T–/P+ results in 54 BM assays. Regarding IG studies, 3 T+/P– and 12 T–/P+ results in 99 BM studies were identified, again using criteria established in methods. Sensitivity and specificity, respectively, were calculated for PB TCR studies (94% and 93%), BM IG studies (71% and 95%), and BM TCR studies (92% and 83%). Analysis of PB *IG* gene rearrangement studies was not performed because of the small number of tests (3; all T–/P–).

Table 2. Immunoglobulin and T-Cell Receptor Gene Rearrangement Results From Peripheral Blood and Bone Marrow				
	PB IG	**PB TCR**	**BM IG**	**BM TCR**
T+/P+	0	17	30	12
T+/P–	0	2	3	7
Total Positive Tests	**0**	**19**	**33**	**19**
T–/P+	0	1	12	1
T–/P–	3	27	54	34
Total Negative Tests	**3**	**28**	**66**	**35**
Total malignant	0	18	42	13
Total nonmalignant	3	29	57	41
Total Tests	**3**	**47**	**99**	**54**

Abbreviations: BM, bone marrow; IG, immunoglobulin; PB, peripheral blood; T+/P+, test positive/phenotype positive; T+/P–, test positive/phenotype negative; T–/P–, test negative/phenotype negative; T–/P+, test negative/phenotype positive; TCR, T-cell receptor.

Characteristics of 12 cases associated with T+/P– *IG* and *TCR* gene rearrangement studies are found in Table 3. The 3 T+/P– IG studies corresponded to nonmalignant diagnoses, including 1 normal marrow, 1 indeterminate result, and 1 result negative for involvement of prior Waldenstrom's macroglobulinemia. Of these 3 patients, 1 later developed acute myeloid leukemia. None of them subsequently developed a lymphoproliferative disorder and the patient with Waldenstrom's was not noted to develop relapse of his disease during the study period. The nine T+/P– TCR results corresponded to 4 normal/nonmalignant pathology reports, 1 negative result for BM involvement of clear cell carcinoma of the kidney, 1 diagnosis of aplastic anemia, 1 diagnosis of polycythemia vera, 1 diagnosis of lymphohistiocytosis, and 1 indeterminate result. Of these patients, 1 subsequently developed acute myeloid leukemia, and none of the patients subsequently developed a lymphoproliferative disorder. For the 12 cases with T+/P– results, the time of follow-up surveillance after the first positive rearrangement study ranged from 23 to 1284 days, with a mean of 363 days and median of 210 days between the initial positive study and last opportunity for surveillance for emergence of a lymphoproliferative disorder. One patient who died from hemophagocytic lymphohistiocytosis shortly after an initial T+/P– gene rearrangement analysis was excluded from this calculation.

Characteristics of 14 cases associated with T–/P+ IG and *TCR* gene rearrangement studies are found in Table 4. The 12 T–/P+ IG studies included 4 cases of multiple myeloma (MM), 3 cases of monoclonal gammopathy of undetermined significance, 1 case of low-grade B-cell non-Hodgkin's lymphoma, 1 case of monoclonal B-cell lymphocytosis, 1 case of low-grade B–non-Hodgkin's lymphoma plus MM, 1 case of low-grade B–non-Hodgkin's lymphomaplus myelodysplastic syndrome, and 1 case of monoclonal gammopathy of undetermined significance plus adult T-cell leukemia/lymphoma. The 2 T–/P+ TCR studies correspond-

Table 3. Characteristics of Test Positive/Phenotype Negative Gene Rearrangement Results				
IG or TCR Testing	Specimen Source	Diagnosis Resulting From Testing	Time to Most Recent Follow-Up	Subsequent Diagnosis
IG	BM	Normal/nonmalignant	650 d	—
IG	BM	Indeterminate	1284 d	AML
IG	BM	No bone marrow involvement of WM of lymph node	364 d	—
TCR	BM	Nonmalignant: HLH	—	—
TCR	BM	No bone marrow involvement of clear cell carcinoma of the kidney	76 d	—
TCR	BM	Normal/nonmalignant	23 d	—
TCR	BM	Normal/nonmalignant	799 d	—
TCR	BM	Myeloproliferative neoplasm: PV	157 d	AML
TCR	BM	Nonmalignant: aplastic anemia	28 d	—
TCR	BM	Normal/nonmalignant	251 d	—
TCR	PB	Normal/nonmalignant	152 d	—
TCR	PB	Indeterminate	210 d	—

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; HLH, hemophagocytic lymphohistiocytosis; IG, immunoglobulin; PB, peripheral blood; PV, polycythemia vera; TCR, T-cell receptor; WM, Waldenstrom's macroglobulinemia.

ed to 1 diagnosis of T-cell acute lymphoblastic leukemia and 1 diagnosis of T-cell large granular lymphocytic leukemia.

DISCUSSION

Results from this study support the general recommendation that PCR-based *IG* and *TCR* gene rearrangement analysis should be interpreted in the context of clinical, morphologic, immunohistochemical, and cytogenetic data in order to establish the diagnosis of a lymphoproliferative disorder. Contrary to our hypothesis, none of the patients with an isolated positive *IG* or *TCR* gene rearrangement result subsequently developed a lymphoproliferative disorder, suggesting these gene rearrangement results were not predictive of a burgeoning malignancy undetected by other tests.

Clonal lymphocyte populations can be detected in nonmalignant settings, leading to T+/P– gene rearrangement results. For example, monoclonal and oligoclonal T-cell populations can be detected by PCR in viral infections, benign skin disorders, advanced age, and recovery from chemotherapy or hematopoietic stem cell transplantation.[7,9–12] Similarly, clonal B-cells can be observed in immunosuppression or autoimmune disease.[13] In the setting of minimal residual disease monitoring, a monoclonal B-cell population may develop additional *IGH* gene rearrangements through the process of clonal evolution, resulting in T+/P– (ie, a new gene rearrangement is detected that appears to be a new monoclonal population but merely represents a new arrangement in the same monoclonal population) or T–/P+ (ie, the presence of new gene rearrangements obscures the original rearrangement associated with the monoclonal population) PCR results.[14]

Table 4. Characteristics of Test Negative/Phenotype Positive Gene Rearrangement Results		
IG or TCR Negative	Specimen Source	Diagnosis Resulting From Testing
IG	BM	MM
IG	BM	MM
IG	BM	MM + low-grade B NHL NOS
IG	BM	MGUS
IG	BM	MM
IG	BM	MBL
IG	BM	Low-grade B NHL NOS
IG	BM	MM
IG	BM	Low-grade B NHL NOS (+ MDS)
IG	BM	MGUS
IG	BM	MGUS (+ ATLL)
IG	BM	MGUS
TCR	BM	T-ALL
TCR	PB	T-LGLL

Abbreviations: ATLL, adult T-cell leukemia/lymphoma; B NHL NOS, B-cell non-Hodgkin lymphoma, not otherwise specified; IG, immunoglobulin; MBL, monoclonal B-cell lymphocytosis; MDS, myelodysplastic syndrome; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; PB, peripheral blood; T-ALL, T-cell acute lymphoblastic leukemia; T-LGLL, T-cell large granular lymphocytic leukemia; TCR, T-cell receptor.

Notably, of the 14 T–/P+ gene rearrangement results observed in this study, 10 (71%) were related to a diagnosis of plasma cell neoplasms. Because plasma cells are B cells, *IG* gene rearrangement analysis can be used to establish clonality in plasma cell neoplasms, such as MM.[15] In rare subtypes of MM, such as nonproducer, nonsecretor MM, *IG* gene rearrangement analysis may at times be the only laboratory assay capable of demonstrating plasma cell monoclonality.[16] Somatic hypermutation of *IGH* genes in postgerminal center B-cell malignancies, including MM is a well-established cause of T–/P+ gene rearrangement analysis,[5,8,17] requiring *IGK* rearrangement analysis to reliably establish clonality in the setting of these diseases.[18] In all 10 T–/P+ cases related to plasma cell neoplasms in the present study, gene rearrangement analysis was performed on *IGH* only. The addition of *IGK* analysis in these cases likely would have decreased the rate of observed T–/P+ results.

Other technical and biological limitations underlie T–/P+ gene rearrangement results. For example, PCR-based gene rearrangement analysis has been shown in some studies to fail to detect monoclonal lymphocyte populations with less than 5% to 10% of clonal cells, and as such, T–/P+ results can be found in the presence of small malignant lymphocyte populations.[5] Occasional T–/P+ also result from the exclusion of select complex gene rearrangements in BIOMED-2 primer sets in order to avoid an unacceptable rate of T+/P– results.[5] Similarly, PCR products falling outside of the 5th or 95th percentiles of size can rarely represent true monoclonal lymphocyte populations despite being regularly excluded from analysis.[19] Last, chromosomal

abnormalities, such as t(11;14) and t(14;18), in B-cell neoplasms are not amplified in *IGH* multiplex PCR and can also cause T–/P+ IG gene rearrangement studies.[6]

This study is subject to important limitations. First, these data were obtained from the electronic medical record of a single clinical site with a primarily male patient population and may not reflect the clinical or laboratory practices of other sites. Additionally, it is possible some patients may have developed a subsequent lymphoproliferative disorder and presented to another hospital than the one examined in this study. Nonetheless, as a chronic care center, we believe our Veterans Affairs facility helps to limit this possibility, as patients seen often maintain some aspect of their care within our system, which would have led to at least updating of their medical problem list (if not frank treatment at our hospital). Last, living patients with positive gene rearrangement results may still develop a lymphoproliferative disorder the time constraints of the study did not allow to be detected, and it may be warranted for us to re-examine the T+/P– patient cohort in 5 years to see if there is any new evidence for development of lymphoproliferative processes.

In summary, the results from the present study suggest positive *IG*/*TCR* gene rearrangement studies are not predictive of lymphoproliferative disorders in the context of otherwise negative BM or PB findings. As such, when faced with equivocal pathology reports, clinicians can be practically advised that isolated positive *IG*/*TCR* gene rearrangement studies do not indicate a need for closer surveillance.

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