A B-Cell Lymphoma Diagnosed in "Floater" Tissue: Implications of the Diagnosis and Resolution of a Laboratory Error

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Abstract: Contamination of a paraffin-embedded tissue block with another patient's tissue can lead to an incorrect diagnosis. We report the use of short tandem repeats for the analysis of DNA extracted from microdissected tissue from unstained slides prepared from a decalcified block from a 33-year-old woman who was previously diagnosed with a low-grade B-cell lymphoma. This diagnosis was based on a single fragment of tissue found among bone fragments taken during orthopedic surgery at a referring hospital. Our results confirm that the B-cell lymphoma tissue was not derived from our patient. Furthermore, we suggest that in cases for which the definitive identification of a tissue contaminant can resolve clinically, therapeutically, and prognostically significant questions, short tandem repeat analysis of DNA derived from microdissected surgical pathology samples should be considered to minimize errors and enhance the quality of care.

Key Indexing Terms: Floater tissue; Short tandem repeats; Microdissection; Analytical errors; Quality assurance. **[Am J Med Sci 2009; 338(3):248–251.]**

Contamination of a paraffin-embedded tissue block with another patient's tissue is a major quality concern issue in surgical pathology and can lead to an incorrect diagnosis. Over the years, several laboratory techniques have been used to distinguish the "floater" tissue from other tissue fragments within the paraffin section. Technological advances employing the use of short tandem repeat (STR) sequences coupled with fluorescent polymerase chain reaction (PCR) and capillary electrophoresis provide a rapid and sensitive technique to discriminate the origin of tissue fragments from different patients.

We report the use of these techniques on microdissected tissue from unstained slides prepared from a decalcified block from a 33-year-old woman who was previously diagnosed with a low-grade B-cell lymphoma. This diagnosis was based on a single fragment of tissue found among bone fragments taken during surgery at the referring hospital. We present convincing evidence that the B-cell lymphoma tissue was not derived from our patient.

Finally, because STR studies are rapid, similar in price to alternative methods, and, most importantly, highly discriminatory, they are increasingly being used at our institution in cases for which the definitive identification of a tissue contaminant can resolve clinically, therapeutically, and prognostically significant questions. This standardized approach will minimize diagnostic errors, ensure accuracy and quality of patient care, and thereby reduce the adverse clinical impact of diagnostic errors.

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CASE REPORT

A 33-year-old woman presented with a fracture of the left humerus. Her history was notable for a large solitary (unicameral) bone cyst of the humerus first resected and repaired in 1992. Over the next 10 to 15 years, she sustained multiple fractures at this same site and underwent numerous surgeries, including autologous bone grafting, to repair this defect.

In 2002, bone fragments taken during rod placement in her left humerus were submitted for routine surgical pathology. The majority of the specimen consisted of devitalized bone and fibrous tissue with scant marrow, showing trilineage hematopoiesis. Unexpectedly, a small (<1 mm^2) separate tissue fragment, composed of a sheet of small lymphocytes, was seen. By immunohistochemistry, these cells were confirmed as B cells that expressed CD20 and CD5, with focal expression of CD23 and no detectable CD10.

The pathologist at the referring institution diagnosed this as a "single, small, detached fragment of soft tissue with apparent involvement by a low-grade, CD5-positive B-cell neoplasm of uncertain clinical significance." In the comment section, the pathologist reported that he "could not entirely exclude the possibility that this soft tissue fragment was actually derived from a different specimen and was artifactually embedded in . . . this case."

The patient was told that she had a low-grade B-cell non-Hodgkin lymphoma and should follow-up with an oncologist. The patient was asymptomatic and chose not to pursue treatment for this diagnosis, although she was followed up on a yearly basis with complete blood counts and physical examination. Five years later, on moving to another city, she sought to establish care with a new hematologist-oncologist for annual follow-up of her presumed lymphoma. Her annual blood counts and examination have repeatedly shown neither leukocytosis nor lymphadenopathy concerning for lymphoma.

After the patient relocated to Nashville, the original pathology material was reviewed, and the original diagnosis of the devitalized bone and fibrous tissue from 1992 was confirmed. The bone reamings from 2002 were also reviewed, and the tissue fragment with the atypical small B-cell population was identified (Figures 1 and 2). The tissue fragment was thought to be a contaminant that had been accidentally embedded into this patient's tissue block.

The patient's new hematologist was contacted regarding the possibility that the tissue on which the patient's diagnosis had been based may not have actually belonged to her. The decision was made to further analyze the material in an attempt to resolve the question of the origin of the tissue fragment

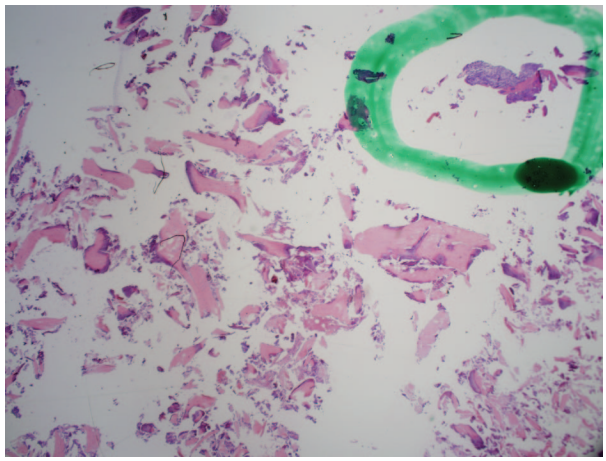

FIGURE 1. Low-power microscopy of the original slide from 2002 bone reamings (H&E, 40×). The tissue fragment in the upper right-hand corner (circled in green) is the lymphoid fragment of interest.

containing an atypical small B-cell population. STR analysis comparing DNA extracted from both the suspicious fragment of tissue from the 2002 biopsy and the patient's peripheral blood leukocytes was suggested. Ten unstained recut slides from the 2002 paraffin block were received for further study.

METHODS

The suspicious tissue fragment was identified on each of the unstained slides using light microscopy and crudely dissected from the surrounding normal bone tissue, using a sterile scalpel. The scrapings were deparaffinized through two 20-minute incubations in xylene and washed with 100% ethanol. DNA was extracted using the Qiagen Puregene DNA Isolation kit (Qiagen, Valencia, CA), following overnight digestion with proteinase K (200 μg/mL). Approximately 5 to 10 ng of DNA was recovered and 2 ng was used for STR analysis. DNA from the patient's peripheral blood was extracted using the Qiagen EZ-1 robot isolation system (Qiagen) and approximately 5 ng was used for analysis. DNA from the B-cell lymphoma tissue fragment and DNA from the patient's peripheral blood were distinguished using PCR amplification for 9 highly polymorphic independently segregating STR loci (AmpFISTR Profiler Plus ID PCR amplification kit Applied Biosystems, Foster City, CA) and 1 gender-specific locus. Amplicons were subjected to capillary electrophoresis on a 3130*xl* Genetic Analyzer (Applied Biosystems), and the unique DNA fingerprint patterns were analyzed using the fragment analysis program GeneMapper v3.7 (Applied Biosystems).

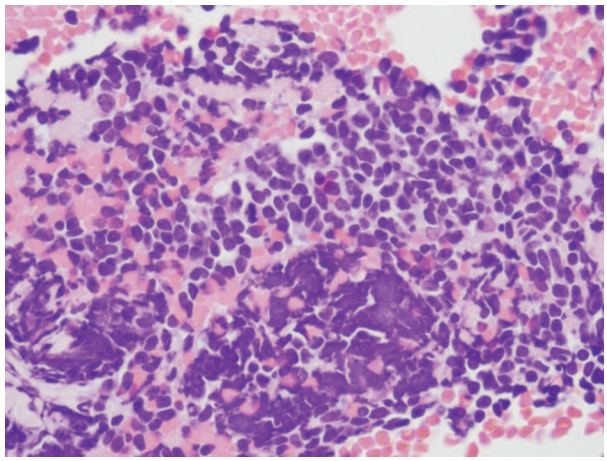

FIGURE 2. High-power microscopy of the lymphoid fragment from the original slide (H&E, 400×).

RESULTS

PCR amplification products, albeit weak, were obtained from the B-cell lymphoma tissue extracted from the 10 unstained slides obtained from decalcified tissue. The genotype for 9 polymorphic loci and a gender-specific locus observed from this DNA was distinguishable from the genotype pattern obtained from peripheral blood DNA from the patient (Table 1; Figure 3). Most notably, the patient is a woman and homozygous for a single peak at 104 base pairs (bp) in length corresponding to DNA amplified from the X chromosome. In contrast, DNA extracted from the B-cell lymphoma tissue yields 2 peaks corresponding to 104- and 110-bp fragments consistent with a DNA pattern obtained from a man representing amplified DNA from the X and Y chromosomes, respectively. In addition to Y chromosome–specific amplicons, DNA from the B-cell lymphoma tissue generated PCR fragments not present in the patient at most of the loci studied (Table 1; Figure 3). Together, these data indicate that the B-cell lymphoma tissue within the left humeral reamings block was not derived from our patient.

DISCUSSION

The possibility of a tissue contaminant resulting in an error in diagnosis is a major quality concern issue in surgical pathology. In one study by Gephardt and Zarbo[1], tissue contaminants were found in up to 2.9% of all surgical pathology samples. More often, the contaminant was on a single slide rather than in the paraffin block. Furthermore, the contaminant was neoplastic in 6.0% to 12.7% of cases, which raises the concern for misdiagnosis of malignancy in these cases. Additionally in a recent report, almost 1% (3 of 335) of pathology medical malpractice claims involved floaters.[2] Over the years, a variety of laboratory methods have been used to discern the provenance of the floater. Blood group antigen immunohistochemistry was initially described[3] as a method for tissue source identification. Other methods such as fluorescence in situ hybridization for sex chromosomes[4] or DNA analysis of a variable number tandem repeats locus[4] have also been used. Although these methods are accurate, each has limited maximal theoretical sensitivities to distinguish any 2 patients (eg, 50/50 chance that any 2 patients are the same sex).

DNA analysis of microsatellites or STR sequences is most commonly used in the clinical pathology laboratory for identity testing to determine the degree of chimerism in specimens from patients who have undergone bone marrow and stem cell transplantation.[5] However, because of the polymorphic nature of these regions, this powerful tool has also been applied to assign incorrectly labeled tissue biopsies,[6] confirm suspected mislabeled clinical laboratory specimens,[7,8] and identify the origin of floater tissue.[9,10] We believe that this case represents the first report of the use of microdissected tissue from unstained slides of a decalcified specimen to determine the origin of a suspected tissue contaminant. The small amount of decalcified tissue available made this case challenging, and could account for it not having been ordered initially. Albeit weak, the genotype pattern obtained from the B-cell lymphoma