Human Pathology (2018) **72**, 52–58

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Adipocyte size variability in benign and malignant lipomatous tumors and morphologic mimics: a quantitative definition using digital pathology☆

Gregory R. Bean MD, PhD*, Kwun Wah Wen MD, PhD, Andrew E. Horvai MD, PhD

Department of Pathology, University of San Francisco (UCSF), San Francisco, CA 94143

Received 21 July 2017; revised 18 October 2017; accepted 25 October 2017

Keywords:
Alt/WDL;
Lipoma;
Adipocyte;
Digital pathology;
Whole-slide scanning;
Image analysis

Summary Among well-differentiated lipomatous lesions, variability in adipocyte size has been proposed as a morphologic feature of malignancy. Specifically, normal adipose tissue and benign lipomas tend to contain adipocytes of uniform size, whereas atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL) is described as containing adipocytes with a conspicuous variation in cell size. However, this proposed variance has never been objectively, quantitatively correlated with diagnosis. Using whole-slide scanning combined with semiautomated digital image analysis, we aimed to quantitatively test the hypothesis that variance in adipocyte size is a feature of malignancy in well-differentiated lipomatous tumors. Whole-slide scanning was performed on representative hematoxylin and eosin–stained slides selected from 130 cases representing benign (lipoma, spindle cell lipoma) and malignant lipomatous neoplasms (ALT/WDL) and morphologic mimics (normal adipose tissue, fat necrosis, fat atrophy). A previously validated, open source software package (Fiji-based Adiposoft) was used in semiautomated analysis of adipocyte size from a representative 1-cm^2 portion of each slide. Median, range, and variance of cell sizes were compared across all diagnoses. Fat atrophy demonstrated smaller adipocyte cell size compared with other diagnoses. Among the remaining diagnostic groups, no significant differences were identified in adipocyte size or variance by objective quantitative morphologic analysis. However, the maximum range of adipocyte size was significantly higher in ALT/WDL than conventional lipoma and spindle cell lipoma. These data quantitate the morphology of ALT/WDL and its mimics and more specifically define the somewhat subjective "variability" of cell size as maximum *range*, rather than *variance*, of adipocyte size.

1. Introduction

The increasing prevalence and importance of molecular pathology have allowed for genetic, epigenetic, proteomic, and metabolomic analyses of specimens, far beyond the morphologic features [1,2]. However, for the pathologist, pattern recognition and detection of minor histologic differences on hematoxylin and eosin (H&E)–stained sections remain the

☆ Disclosures: The authors have no conflicts of interest to disclose. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

* Corresponding author at: Department of Pathology, University of California San Francisco (UCSF), 1825 4th St, M2372, San Francisco, CA 94143.
E-mail address: gregory.bean@ucsf.edu (G. R. Bean).

https://doi.org/10.1016/j.humpath.2017.10.030
0046-8177/

mainstay of diagnostic pathology. Yet morphologic criteria used to accurately differentiate benign from malignant tumors are often subjective and poorly reproducible. The application of computational image analysis to histologic sections is one approach to yield an unbiased quantitative evaluation and to minimize interobserver variability and interpretation. Digital methods have been successfully used for quantification of such features as mitotic index, cellularity, and immunohistochemical staining of biomarkers [3-6]. Quantitative analysis can, therefore, be a useful tool to reinforce or, alternatively, refute morphologic features claimed to be diagnostically useful. The quantitative and reproducible nature of digital morphometry also provides information about the *magnitude* that a given morphologic variable differs between diagnostic entities. The latter can be helpful to decide which morphologic features may be easiest to recognize using routine microscopy.

Lipomas, comprised predominantly of mature adipocytes, can be histologically indistinguishable from nonlesional white adipose tissue. It is often the clinical history or gross evaluation of a mass that reveals its neoplastic nature. Malignant lipomatous tumors include atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL), dedifferentiated liposarcoma, myxoid/round cell liposarcoma, and pleomorphic liposarcoma. The morphologic appearance of lipoma and ALT/WDL can also be remarkably similar; select sampling of ALT/WDL may show identical histology to that of a benign lipoma. The presence of fat necrosis can be an added variable that imparts worrisome histologic features; the constellation of increased cellularity, vacuolated histiocytes, multinucleated giant cells, and enlarged necrotic adipocytes may be mistaken for malignancy. Often associated with cachexia, fat atrophy is usually described as having smaller adipocytes than normal fat or ALT/WDL, but variability of adipocyte size is seldom mentioned in the description of this entity. For the pathologist, awareness of the clinical history including patient age, history (trauma, weight loss), lesional site, and depth, as well adequate sampling, is necessary to make a correct diagnosis. In challenging cases, a variety of ancillary techniques including immunohistochemistry and molecular and genetic tests have become widely used to confirm or exclude a diagnosis of ALT/WDL [7-11]. However, the expense and time of adjuvant tests are not negligible.

The current World Health Organization edition characterizes ALT/WDL as "showing significant variation in cell size and at least focal nuclear atypia in both adipocytes and stromal cells" [12]. In contrast, lipoma is reported to demonstrate uniformly sized adipocytes. Adipocyte size variability of ALT/WDL is pervasive in the sarcoma literature [13-16], yet no published reports interrogate this concept objectively. In the past, there had been no easy way to rapidly, accurately, and reproducibly measure the size of intact adipocytes on histologic sections. Older techniques relied on collagenase digestion, mesh separation, and/or centrifugation to isolate individual adipocytes, which were subsequently stained and analyzed by hemocytometer or flow cytometry [17-19]. Such tissue disruption, however, can lead to cell loss and does not accurately maintain the histopathological context. Alternatively, histological images could be captured from H&E—stained sections followed by manual analysis. The number of adipocytes can be counted and the diameter measured, from which the average cell volume could be derived, but such a technique is extremely laborious and impractical.

With the technological advent of faster and higher-resolution digital scanners, whole-slide digital imaging can be used in concert with recent advances in quantitative histomorphometry. Both now allow for semiautomated image analysis of microscopic sections, offering a simpler and rapid alternative to analyzing cell size [20-22]. A number of tools have been designed and reported to quantitate cell size. One of these, Adiposoft, is an open source, Java-based plugin for the freely available software package Fiji [21]. Adiposoft has been validated with comparisons to both the analysis of cells in suspension and the gold standard of manual computation. Using filtering and thresholding methods, a seeded watershed algorithm is applied that reinforces cellular boundaries. The software counts each bounded space within predefined sizes and creates an output file in .xls format. Compared with other methods, Adiposoft provides similarly accurate results while being less costly and consuming less time and effort [21].

We paired whole-slide scanning with semiautomated image analysis to examine cell size in benign and malignant lipomatous tumors, as well as normal adipose tissue, fat necrosis, and fat atrophy. The present study tests the hypothesis of whether variability in cell size correlates with malignancy in well-differentiated adipocytic tumors. A second, more general goal was to test the feasibility of translating subjective histomorphologic criteria into quantitative variables.

2. Materials and methods

2.1. Study population

This study was approved by the institutional review board of the University of California San Francisco (UCSF). Cases of normal fibroadipose tissue (n = 23), fat necrosis (n = 15), fat atrophy (n = 17), lipoma (n = 21), spindle cell lipoma (n = 21), and ALT/WDL (n = 33) were identified by examining excision specimens and autopsies from the archives of the Department of Pathology at UCSF spanning the years 1998 to 2015. These diagnoses were confirmed by an expert in soft tissue pathology (A. E. H.) using a combination of clinical, routine H&E, immunohistochemical, and genetic findings.

2.2. Whole-slide scanning and image analysis

One representative H&E-stained slide from each case was chosen, and whole-slide scanning was performed at 20× viewing magnification using an Aperio XT scanner. In all excision specimens, the slides contained at least 4 cm^2 of pure tissue of interest. Some fat atrophy cases were more focal, as they were