

# Atomic force microscopy: a promising aid in diagnosis of uterine smooth muscle neoplasms



**OBJECTIVE:** Malignancy diagnosis of uterine smooth muscle neoplasms can be challenging. Morphologic features are subjective, and the utility of immunohistochemistry is still debated.<sup>1,2</sup> We propose to characterize and compare the ultrastructural and mechanical properties of leiomyoma and leiomyosarcoma with those of normal myometrium, using atomic force microscopy (AFM) technique.

**STUDY DESIGN:** Three representative groups of samples were selected from the database of the Pathology Unit—San Martino Hospital, Genoa, Italy. Group 1 comprised 1 sample of normal myometrium and 1 sample of spindle cells leiomyoma from a 39-year-old patient and 1 sample of spindle cells leiomyosarcoma from a 62-year-old patient. Group 2 comprised 1 sample of normal myometrium and 1 sample of spindle cells leiomyoma from a 49-year-old patient and 1 sample of spindle cells leiomyosarcoma from a 67-year-old patient. Group 3 comprised 1 sample of normal myometrium and 1 sample of spindle cells leiomyoma from a 50-year-old patient and 1 sample of spindle cells leiomyosarcoma from a 55-year-old patient. Two shadowed sections (thickness of 5  $\mu\text{m}$  for AFM-imaging and 15  $\mu\text{m}$  for AFM-indentation testing) were collected separately from each paraffin-embedded tissue specimen. After being removed from the paraffin and dried, the specimens were left exposed for AFM-scanning. Sections were not stained.

AFM-imaging was performed in contact mode at room temperature and in air on regions of interest, which were selected accurately through optical microscopy. The specimens for mechanical characterization were probed in liquid conservation medium (phosphate-buffered saline solution) at room temperature through AFM-nanoindentation on three  $32 \times 32$ -array/ $90 \times 90$ - $\mu\text{m}^2$  regions of interest. Image processing was made with the use of Fiji (<https://doi.org/10.1038/nmeth.2019>). Elastic modulus values that were calculated from the AFM-indentation test were analyzed statistically ( $P \leq .05$ ) with the use of MedCalc software (version 18.11; Ostend, Belgium).

**RESULTS:** AFM-imaging evidences regularly oriented smooth muscle cells in normal myometrium. The nuclear pattern shows blunt-ended, thin ellipsoidal nuclei, centrally located in sarcoplasm. Larger myofibrils and smaller connective fibrils show similar positioning, bestowing high homogeneity to the interstitium (Figure 1, A). Similarities in regular orientation of smooth muscle cells, nuclei density, shape, and location in the sarcoplasm were observed between leiomyoma and normal myometrium.

However, despite such similar directionality, myofibrils are narrower, and the interstitium is comparatively wider and devoid of small connective fibrils (Figure 1, B). Contrarily, leiomyosarcoma evidences irregularly oriented pleomorphic smooth muscle cells. Nuclei appear bigger ( $>50\%$  of sarcoplasm), more rounded, and at a higher density than in leiomyoma and normal myometrium. Interstitium shows further loss of myofibrils and small connective fibrils that are replaced by amorphous, nonfibrillar material. This feature confers high irregularity and heterogeneity to the tissue ultrastructure (Figure 1, C).

Concerning the AFM-indentation measurements, which were performed on each group of samples, The analysis of variance assay test indicates statistically significant differences between the elastic moduli of different samples ( $P < .0001$ ), and a *t*-test showed a significantly lower average elastic modulus (ie, softer tissue) for leiomyosarcoma when compared with leiomyoma and an even lower average elastic modulus when compared with normal myometrium (Figure 1, D). Interestingly, nearly 80% of elastic modulus values that are measured on different points of leiomyoma and  $>95\%$  of those measured in leiomyosarcoma are below the mean elastic value  $\bar{E}$  of the normal myometrium (Figure 1, E).

**CONCLUSION:** Leiomyoma, leiomyosarcoma, and normal myometrium show significant differences at the nanoscale that cannot be observed easily and measured at the microscale with clinically available techniques. Ultrastructural differences in myofibrils positioning and thickness, nuclear pattern, and interstitium are observed clearly. Similar to other tumor types, elastic modulus of tumorous myometrium decreases compared with normal myometrium.<sup>3–5</sup>

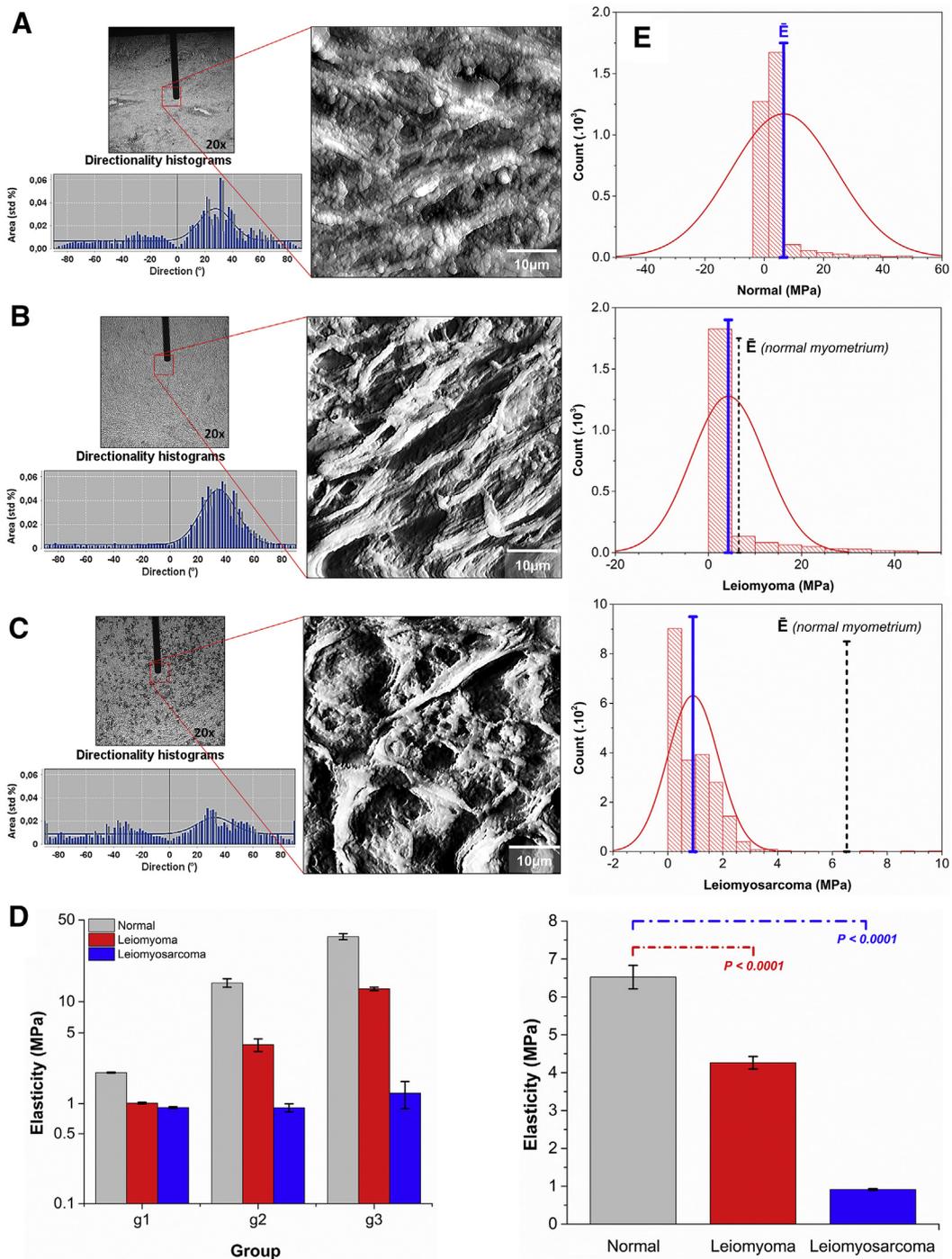
Based on these preliminary results, we propose AFM as a technique capable of providing complementary and useful morpho-mechanical parameters in terms of differential diagnosis in gynecopathology. ■

Leonardo A. Peñuela, MD, PhD  
Ezio Fulcheri, MD  
Fetal-Placental and Perinatal Unit  
Giannina Gaslini Institute  
DiNOGMI  
University of Genoa  
Genoa, Italy

Valerio G. Vellone, MD, PhD  
Roberto Fiocca, MD  
Pathology Unit  
San Martino Hospital

## FIGURE

## Atomic force microscopy ultrastructural and mechanical characterization



**A**, Normal myometrium; **B**, leiomyoma; **C**, leiomyosarcoma. *Left column*, Optical microscopy images (the *red squares* are the selected regions of interest for atomic force microscopy scanning); histograms of the preferential direction calculated for the corresponding atomic force microscopy image. *Right column*, Representative  $50 \times 50 \mu\text{m}^2$  atomic force microscopy topography images of the selected region of interest. **D**, *Left plot*: Average elastic modulus calculated from atomic force microscopy—nanoindentation measurements on each sample of groups. *Right plot*: Cumulative average elastic modulus of groups classified by type of tissue, (error bars: 1 standard error of the mean). **E**, Distribution of the measured elastic moduli for each sample;  $\bar{E}$  represents the average elastic modulus of the normal myometrium.

MPa, megapascal.

Peñuela. Atomic force microscopy. *Am J Obstet Gynecol* 2019.

DISC  
University of Genoa  
Genoa, Italy

Gian M. Vigliercio, MD  
Medicine School  
University of Genoa  
Genoa, Italy

Roberto Raiteri, EE, PhD  
Department of Biomedical Engineering, Robotics and Systems  
DIBRIS  
University of Genoa  
Genoa, Italy

Simone Ferrero, MD, PhD  
Department of Gynecology and Obstetrics  
DiNOGMI  
University of Genoa  
Genoa, Italy

The authors report no conflict of interest.

REFERENCES

1. Oliva E. Practical issues in uterine pathology from banal to bewildering: The remarkable spectrum of smooth muscle neoplasia. *Mod Pathol* 2016;29(suppl1):S104–20.
2. Ferenczy A, Richart RM, Okagaki T. A comparative ultrastructural study of leiomyosarcoma, cellular leiomyoma, and leiomyoma of the uterus. *Cancer* 1971;28:1004–18.
3. Sokolov I. Atomic force microscopy in cancer cell research. In: Nalwa HS, Webster T, eds. *Cancer nanotechnology-nanomaterials for cancer diagnosis and therapy*. NY: American Scientific Publishers; 2006: 43–59.
4. Zemla J, Danilkiewicz J, Orzechowska B, Pabijan J, Seweryn S, Lekka M. Atomic force microscopy as a tool for assessing the cellular elasticity and adhesiveness to identify cancer cells and tissues. *Semin Cell Dev Biol* 2018;73:115–24.
5. Cui Y, Zhang X, You K, et al. Nanomechanical characteristics of cervical cancer and cervical intraepithelial neoplasia revealed by atomic force microscopy. *Med Sci Monit* 2017;23:4205–13.

© 2019 Elsevier Inc. All rights reserved. <https://doi.org/10.1016/j.ajog.2019.05.013>

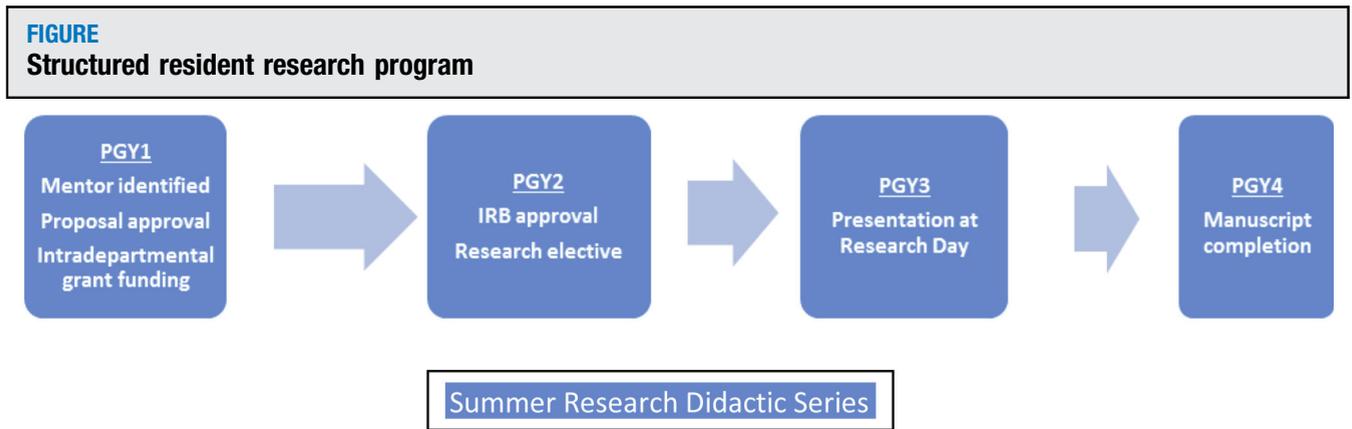
# Impact of a structured obstetrics and gynecology residency research program



**OBJECTIVE:** The Accreditation Council on Graduate Medical Education requires that all obstetrics and gynecology residency programs ensure resident participation in scholarly activities.<sup>1</sup> A previous national obstetrics and gynecology program director survey demonstrated that 95% require research projects and that successful programs often had a research rotation and/or a formal research curriculum.<sup>2</sup> Other studies specific to obstetrics and gynecology residency programs have highlighted other mechanisms to improve productivity including a biweekly research workgroup<sup>3</sup> and research study teams.<sup>4</sup> At the University of Colorado obstetrics and gynecology residency program, we instituted a formal research

curriculum in 2013 (Figure) after the appointment of 2 Assistant Residency Program Directors (APDs). We also incorporated a summer research didactic curriculum and introduced an internal departmental grant funding mechanism. In this study, we determined the impact of this structured program on scholarly productivity as defined by the number of presented posters or oral abstracts at national conferences and published peer-reviewed manuscripts.

**STUDY DESIGN:** Because the program was initiated in 2013, we defined the preintervention group as those who graduated between 2012 and 2014 (n=27); the postintervention group was



Description of timeline for resident research program at the University of Colorado.

IRB, Institutional Review Board; PGY, Postgraduate year.

Guiahi. Structured residency research program. *Am J Obstet Gynecol* 2019.