Clinics in Surgery

Case Report
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Endometriosis: Diagnosis Procedure on Peritoneal Washing

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Abstract

The challenge for endometriosis is to find a non-invasive diagnostic technique, early before the progression of the disease, to preserve fertility, improve comfort of life and finally avoid pelvic complications. Culdocentesis with washing easily allows exploring polymorphic peritoneal cellularity. We propose an immunohistochemical investigation procedure on cytoblocks allowing sharing of different cellular categories of the peritoneal fluid.

Keywords: Endometriosis; Peritoneal washing; Culdocentesis; Laparoscopy; Peritoneal fluid

Case Presentation

The ectopic endometrium disseminated or implanted in the peritoneal cavity, develops very varied histological forms in more or less extensive anatomical locations. It can share various characteristics related to the progression of the disease, and to biological and endocrine environment or treatment. This explains the clinical and biological polymorphism of this pathology. An endometrial cellularity in the peritoneal fluid represents by itself an important diagnostic signature of the disease and as it supposes a process of diffusion, a potential cause of resurgence. It probably looks more like an ascitic cell culture than a solid-shaped tissue contamination. It is therefore of the greatest interest to document this peritoneal cellular behavior. Moreover, this identification is also of decisive importance in the period of fertility as the progestatives, often implemented as palliatives, are incompatible with a pregnancy project. Cytopathologists routinely look for cellular elements in the peritoneal washings to establish, among others, the diagnosis of endometriosis [1-5]. Some indirect arguments are noteworthy. Siderophagus or hemolysed blood or three-dimensional cell groups are suggestive of endometriosis. However, the cytological characters readable on the sedimentation or apposition techniques do not make it reliably possible to carry out the diagnosis. Most often cellular clusters observed in the washing liquids do not permit to certify the diagnostic, whereas in contrast biopsy specimens are positive. Epithelial cell clusters are associated with many varieties of reactive cells, mesothelial plaques, inflammatory cells and various epithelial cells, whose origin is uncertain. Some authors have already pointed out the interest of cytoblocks complemented by an immunohistochemical phenotyping to clarify the diagnosis [6,7]. We propose an immunohistochemical investigation procedure on cytoblocks allowing sharing of different cellular categories of the peritoneal fluid. We wish to illustrate the precise phenotypic characters that make it possible to reduce the pitfalls of these investigations.

Methods

Peritoneal washes performed by culdocentesis or laparoscopy are examined before biopsies taken in the context of endometriosis investigation. Peritoneal fluid fresh or kept for 24 h at + 4°, representing a volume between 100 and 200 ml, is centrifuged 2500 rpm 5 min to obtain a pellet. Supernatant is removed. The pellet fixed 20 ml in alcohol 100°, 20 min. Alcohol is removed. Two drops of each component Shandon™ Cytoblock™ Cell Block Preparation System poured on the pellet, provide an aggregate of cohesive cells, cell cluster placed in a cassette, transferred in 70° alcohol for formalin fixation one or 2 h followed by paraffin embedding according to a standard histological technique. A standard histological staining identifies all cell clusters. Immunohistochemistry performed with differential markers objectives the cellular categories represented in the peritoneal fluid Table 1 and 2. The cytoblock processing makes it possible to identify in the peritoneal fluid histological motifs of endometriosis at various stages and periods. There are fragments of complete endometrial mucosa, authenticated by simple morphology. These fragments sometimes appear dissociated. This is the case in laparoscopic washing fluids. This endometriosis component scan

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Table 1: Immunohistochemical process.

Antibodies	Clones	Dilution	Provider	Automaton	Chromogen
CD 68	PG-M1	1/200	DAKO	Leica Bond-Max*	DAB
Calretinin	DAK-Calret 1	1/100	DAKO	Leica Bond-Max	DAB
Pax 8	MRQ-50	1/100	Cell Marque	Leica Bond-Max	DAB
Vimentine	V9	½ prediluted	DAKO	Leica Bond-Max	DAB
WT1	6F-H2	1/50	Cell Marque	Leica Bond-Max	DAB
CD 10	SP 7	1/1 Ready To Use	Roche	Benchmark ultra, Ventana**	DAB

Phenotypes identification: CD68: Macrophages; PAX8: Müllerian Type Cells; WT1: Tubal Cells; Calretinin: Mesothelial Cells Vimentin; Endometrial Epithelial Cells; CD10: Endometrial Stroma

* Detection Leica Bond-Max: Bond Polymer Refine Detection

** Detection Benchmark Ultra Ventana**:** OptiView DAB IHC Detection Kit; 1 Rotina 46 Hettich

Table 2: Phenotype of different cellular categories in the peritoneal fluid.

Endometrium Epithelial Chorion	Peritoneal mesothelial cells	Tubal epithelial cells
Vimentin+ Vimentin+	Vimentine -	**Vimentine +**
Pax 8 + CD10+	CD 10 -	**Pax 8 + CD 10 -**
Calretinin–	**Calretinin +**	Calretinin -
WT 1 -	WT1 -	**WT1 +**

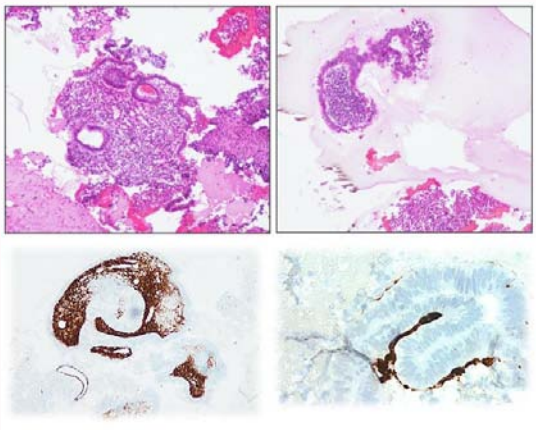

Figure 1: Endodométrial fragments: laparoscopic peritoneal washing.

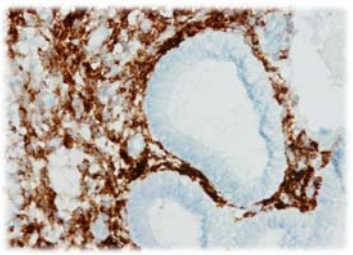

Figure 2: CD10.

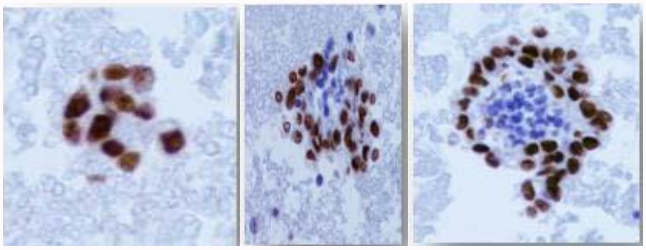

Figure 3: Various sizes of epithelial of endometriosis clusters in peritoneal fluid PAX 8+.

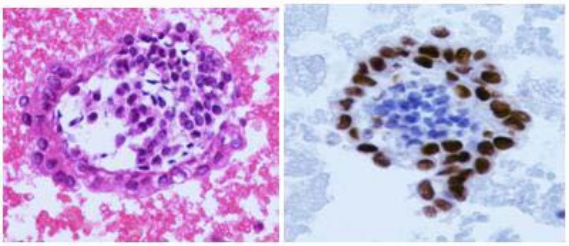

Figure 4: Sphere of cells in peritoneal fluidepithelial corona PAX 8+.

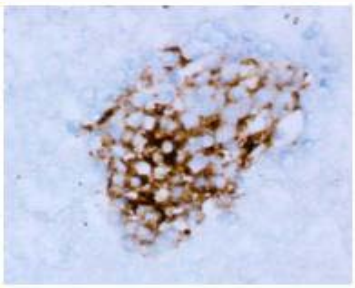

Figure 5: Chorion CD10+.

also organizes in a new mode that represents a form of culture adaptation. Gradually epithelial crowns tend to organize around the chorion. Other motifs are present; there may be small isolated clusters of epithelial cells, clusters of epithelial cells centered on chorion and true small morules which morphology correspond to organotypic culture. It makes also possible to carry out on these blocks other investigations on the phenotypic profile. Steroidal receptors prove very heterogeneous. Phenotypic modifications corresponding to a form of epitheliomesenchymal transition also occur with the partial loss of the cytokeratin 7 expression.

Illustrations

These phenotypes allow identifying the most cellular populations represented in the peritoneal fluid. Moreover, their association is exclusive of various cell types. The antibodies are available and used daily in gynecological pathology (Figures 1-11).

Discussion

Noninvasive methods for accurately documenting the progressive form of endometriosis are virtually non-existent. Investigations on the eutopic endometrium are not performing routinely. Decisive

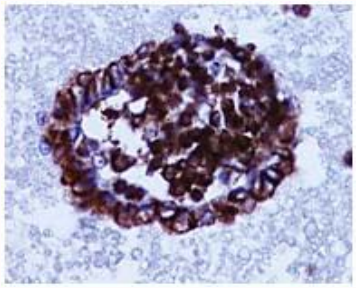

Figure 6: Vim+: Chorion and epithelial.

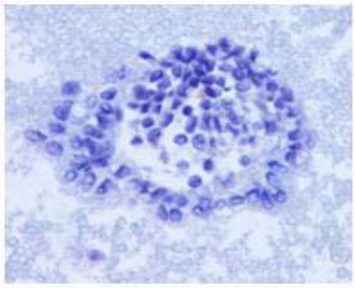

Figure 7: Calretinin negative.

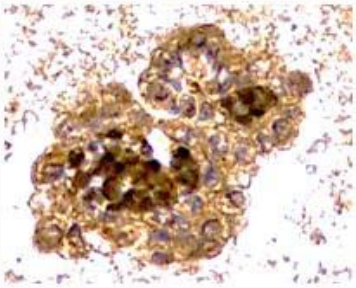

Figure 8: Nuclear WT 1 negative.

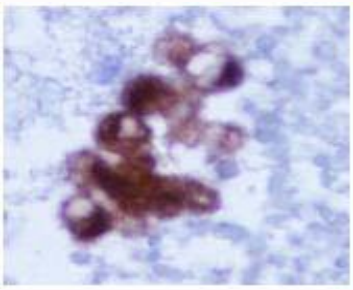

Figure 9: Mesothelialcells Calret +.

indication for endoscopic explorations requiring anesthesia and hospitalization ought to be strongly documented. Peritoneal washing by culdocentesis followed by a phenotypic investigation provides evidence of the disease. It is less invasive than laparoscopy to follow up. Moreover, this technique allows many differentials with the tumor pathology [8,9]. The immunohistochemical phenotype is similar to the protocol published by Dorien et al. [10]. However, this publication was oriented to verify Sampson's theory about retrograde menstruation. Our observations concern histologically documented endometriosis, whose endometrial population in peritoneal fluid,

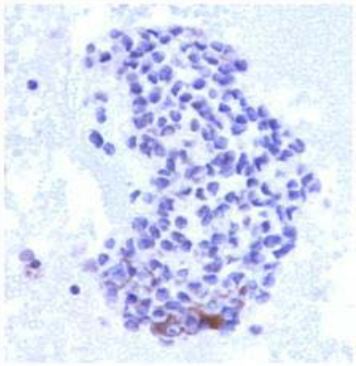

Figure 10: Chorion: PAX 8 negative.

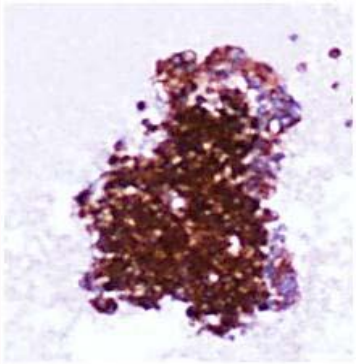

Figure 11: Vimentine positive (chorion).

is representative of an established peritoneal endometriosis. It is important to consider the meaning of the different cluster of cells, in particular those representatives of an organotypic cell culture. The cytoblock procedure represents a reliable method open to comparatives genomic and proteomic studies.

Conclusion

Peritoneal washing, performed by laparoscopy or Culdocentesis followed by cytoblock inclusion, completed with a precise phenotypic investigation, is a powerful tool. It leads to the objective diagnosis of endometriosis. These forms are relevant for endoscopic investigation or surgery. Surgery is contributive to remove adverseness lesions as endometriomas, in a period of life where the women are concerned to regain their fertility in the short term. This methodology is to be associated with a predictive profile giving precious indications to endocrine treatments.

References

1. Cantley RL. The role of peritoneal washings in the diagnosis of endometriosis. Diagn Cytopathol. 2018;46(5):447-51.
2. Zuna RE, Mitchell ML. Cytologic findings in peritoneal washings associated with benign gynecologic disease. Acta Cytol. 1988;32:139-47.
3. Potuono JA, Herran C, Echanojauregui AD, Riego AG. Peritoneal washing and biopsy in laparoscopically diagnosed endometriosis. Fertil Steril. 1982;38:538-41.
4. Stowell SB, Wiley CM, Perez-reyes N, Powers CN. Cytologic diagnosis of peritoneal fluids. Applicability to the laparoscopic diagnosis of endometriosis. Acta Cytol 1997;41:817-22.
5. Barkan GA. Morphologic features of endometriosis in various types of

cytologic specimens. Diagn Cytopathol. 2013.

6. Selvaggi SM. Diagnostic pitfalls of peritoneal washing cytology and the role of cellblocks in their diagnosis. Diagn Cytopathol. 2003;28:335-41.
7. Dharan M. The adjuctive value of CD 10 immunostaning on cell block preparation in pelvic endometriosis. Acta Cytol. 2009;53(6):625-9.
8. Markuly SN, Fanning J, Hindman TL, Galle PC, Ertmoed E, Hilgers RD. False-positive malignant peritoneal cytology associated with pelvic endometriosis. J Am Osteopath Assoc. 1994;94(2):157-61.
9. Peritoneal Washings. Edmund S. Cibas, in Cytology (Third Edition), 2009.
10. Dorien FO. The presence of endometrial cells in peritoneal fluid of women with and without endometriosis. Reprod Sci. 2017;24(2):242-51.