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ORIGINAL RESEARCH ARTICLE

Histopathological examination of the ectocervical biopsy in non-transplanted uteri: A study contributing to the provisional scoring system of subclinical graft rejection after uterus transplantation

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Abstract

Introduction: Uterus transplantation is a causal treatment for absolute uterine factor infertility. Assessing rejection signs using a histopathological examination of the ectocervical biopsy from the transplanted uterus is common practice in all human uterus transplants worldwide to date. A provisional scoring system was used for the histopathological assessment of subclinical rejection signs in uterus recipients. Here we hypothesized that histopathological and immunohistochemical findings in the normal uteri would differ from the borderline category of subclinical rejection in uterine transplants.

Material and methods: This prospective observational study included ectocervical biopsies of 54 women who underwent hysterectomy for benign reasons. All biopsy samples were assessed histopathologically and immunohistochemically.

Results: Most of the ectocervical biopsies showed clustering lymphocytic infiltrates affecting the stromal–epithelial interface with the epithelial influx of lymphocytes, primarily CD45RO-positive activated T-cells with CD8 T-lymphocyte predominance. CD4-positive T-lymphocytes and B-cells were rarely detected in the ectocervix. These morphological findings and immunoprofiles of lymphocytic populations overlapped with the so-called borderline changes defined in the provisional scoring system for rejection in the transplanted uteri. The immunoprofiles of ectocervical and endocervical lymphocytic populations differed, with strikingly prominent B-cell participation in the endocervix vs the rare detection of B-cells in the ectocervix.

Conclusions: The histopathological and immunohistochemical findings in the uteri of premenopausal women were similar to the borderline category of the currently used provisional scoring system of subclinical uterine rejection utilized in all uterine transplant studies. However, future similar studies are required to validate our findings.

Abbreviations: H&E, hematoxylin and eosin; UTx, uterine transplantation.

KEYWORDS
cervical biopsy, histopathology, hysterectomy, immunohistochemistry, organ rejection, uterus transplantation

1 | INTRODUCTION

The first human uterus transplantation (UTx) trial for the treatment of absolute uterine factor infertility began with procurement and transplant procedures using the living donor concept. Since then, this experimental treatment method has undergone significant progress and expansion.[1,2] In late 2014, the first-ever child after living donor UTx was born in Gothenburg, Sweden, followed by several others, including two uterine recipients having two offspring each.[3,4] The first Swedish UTx study commenced in 2012–2013 and was the first-ever concluded human experimental trial worldwide.[5] In late 2017, the first childbirth after deceased donor UTx was achieved in Sao Paolo, Brazil.[6] The onset of the Czech mixed living and deceased donor UTx trial was preceded by a UTx study of interest in a group of women with congenital agenesis of the uterus in whom a neovagina was surgically created using laparoscopic Vecchietti's vaginoplasty.[7] The surgical results of the nine Czech UTx cases were published in 2019 and the preliminary assisted reproductive outcomes were recently reported.[8,9]

Several aspects of UTx remain under investigation, including monitoring of the signs of uterine graft rejection after transplantation and during pregnancy. Given the lack of less invasive methods to control UTx rejection, the current practice is based on regular ectocervical biopsies and their histopathological evaluation according to the provisional scoring system of uterine rejection suggested by Mölne et al.[10] Based on this classification, histopathological rejection of the uterine graft is divided into mild, moderate and severe grades, and a borderline change category. It is currently uncertain whether the above changes seen in the uterine ectocervix correspond to changes occurring in the entire transplanted uterus. However, based on the detailed histological assessment of the seven uterine explants, the Swedish pioneers in UTx research recently reported that the inflammatory changes in the uterine cervix represent those throughout the entire uterus; therefore, ectocervical biopsy seems suitable for allograft control.[5]Here we report analyses of histopathological and immunohistochemical findings of ectocervical biopsies among a group of healthy premenopausal women who underwent hysterectomy. We further aimed to compare our findings with the scoring system for subclinical rejection in women with transplanted uteri.

Key message

The study demonstrated that the histopathological and immunohistochemical findings of the majority of ectocervical biopsies from the uteri of premenopausal women were similar to the borderline category of the provisional scoring system of subclinical graft rejection after uterine transplantation.

2 | MATERIAL AND METHODS

A total of 54 premenopausal women with benign uterine conditions such as uterine leiomyoma ($n = 25$) and abnormal uterine bleeding ($n = 29$) confirmed by hysteroscopy who were scheduled for an abdominal or laparoscopic-assisted vaginal hysterectomy at our obstetrics and gynecology department were included in this prospective non-randomized study. We aimed to perform an ectocervical biopsy without colposcopy magnification at the start of surgery under general anesthesia to minimize the morbidity of the participants related to an otherwise painful cervical biopsy. A biopsy was performed close to the transformation zone and the cervical canal from the anterior portion of the uterine cervix.

The inclusion criteria for the study participants were as follows: premenopausal age, non-malignant indication for surgery, normal preoperative cervical Papanicolaou smear findings, no vaginal or cervical infection at the time of biopsy, no perioperative uterine bleeding, no immunological or autoimmune disease, no immunosuppressant use, and no use of corticosteroids or any medication altering the immunological status. Informed consent was obtained from each participant after we explained the principles and aims of the trial and the risks related to ectocervical biopsies. The participants' main demographic characteristics were documented.

The study hypothesis was that histopathological and immunohistochemical findings of the normal uteri would differ from the borderline categories of rejection of uterine transplants suggested as an analogy to the rejection classifications for other organ transplants to avoid overtreatment by antirejection therapy. Borderline changes in transplanted uteri are histopathologically characterized as a few small and nonconfluent, at least two nested foci of inflammation, predominantly with lymphocytes in the epithelial–stromal interface, and frequently accompanied by intercellular edema. Minimal inflammation in the papillary stroma can also be detected.[10] The morphological findings of the cervix in the proliferation and secretion phases of the menstrual cycle were also compared, particularly with respect to cellular infiltrates in the epithelial–stromal interface and the cervical stroma.

2.1 | Histopathological examination

Biopsy samples (3–4 × 6–8 mm) were fixed in neutral buffered 4% formaldehyde, transported to the histopathological laboratory, postfixed and embedded in paraffin. Subsequently, the paraffin blocks were sectioned into 4-μm-thick histological sections and

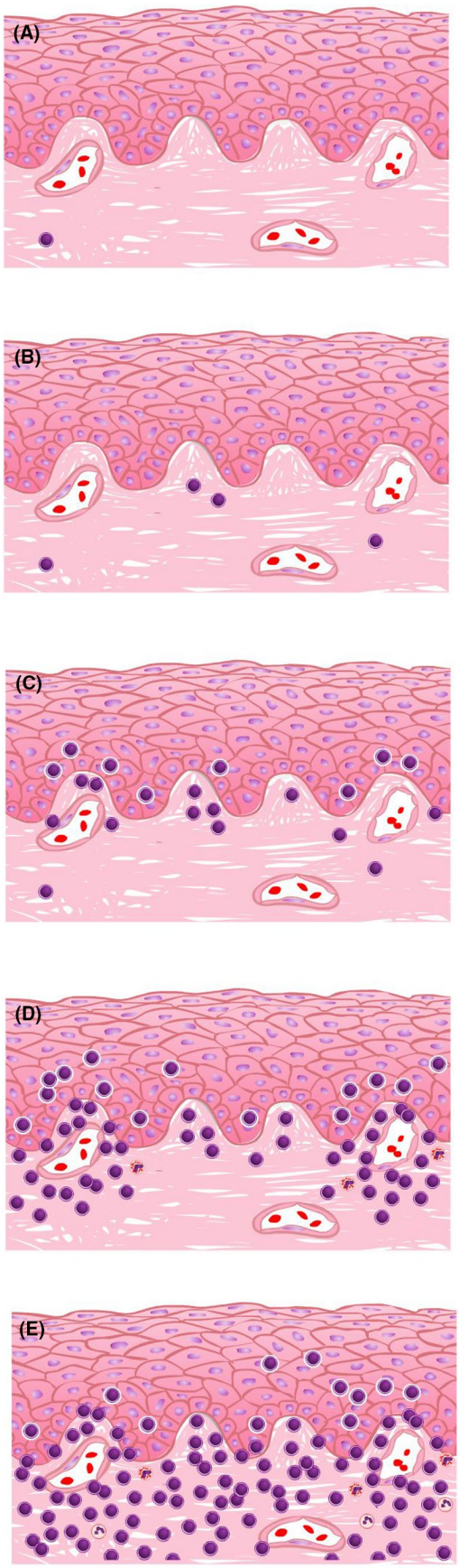


FIGURE 1 The semiquantitative scoring system proposed by the study. (A) Category 1: No or up to 20 isolated lymphocytes in the whole biopsy sample. (B) Category 2: Scattered cells with more than 20 sparse lymphocytes but without clustering within the biopsy sample. (C) Category 3: Clustered cells containing lymphocytes tended to form small clusters seen mainly at the epithelial–stromal interface. (D) Category 4: Focal infiltrates with lymphocytes tending to form large nests or lymphatic follicles. (E) Category 5: Diffuse infiltrates with continuous inflammatory infiltrates within the tissue

stained with hematoxylin and eosin (H&E) and Masson's trichrome staining (to differentiate collagen fibers and fibrosis foci, including intimal sclerosis of the arteries). The histopathological examination focused on the epithelial–stromal interface and cellular infiltration into the cervical stroma. Cervical biopsies were assessed for the presence of inflammatory infiltrates (including interface and microvascular and perivascular stromal inflammation), arteriopathy (including endothelialitis and intimal sclerosis) and epithelial dysplasia. All samples were evaluated by two pathologists specializing in gynecological and lung and heart transplantations with experience in histological assessment of cervical biopsies of transplanted uteri.

2.2 | Immunohistochemistry

The biopsies were immunostained for CD45RO, CD8, CD4, CD20 and C4d, particularly to characterize cellular infiltration. Thin histological sections (3 μm thick) were used, and each sample was stained using the following antibodies and protocols: anti-CD45RO antibody (clone UCHL1 [Agilent–Dako, Santa Clara, CA, USA], dilution 1:300, pretreatment by heating in a buffer solution of pH 6 in a water bath); anti-CD8 antibody (clone C8/144B-[Agilent], dilution 1:200, pretreatment by heating in a buffer solution of pH 9 in a water bath); anti-CD4 antibody (clone 4B12 [BioGenex Laboratories, Fremont, CA, USA], dilution 1:250, pretreatment by heating in a buffer solution of pH 9 in a water bath); anti-CD20 antibody (clone L26 [Agilent], dilution 1:300, pretreatment by heating in a buffer solution of pH 6 in a water bath); anti-C4d antibody (clone A24-T [Zytomed Systems GmbH, Berlin, Germany], dilution 1:150, pretreatment by heating in a buffer solution of pH 6 in a water bath). The detection was performed using a one-step micropolymeric non-biotin system (Bio SB-- Bioscience for the World, Santa Barbara, CA, USA) with a peroxidase complex and 3,3′-diaminobenzidine tetrahydrochloride. The nuclei were counterstained with hematoxylin.

2.3 | Semiquantitative scoring system

To characterize lymphocyte populations using H&E staining and immunohistochemistry, a semiquantitative scoring system was used instead

of absolute cell count to increase the reproducibility of the routine biopsy evaluations. The five-tier score was assessed (Figure 1) as follows.

Category 1: None or up to 20 isolated lymphocytes within the entire biopsy sample (number of lymphocytes corresponding to the normal cervical biopsy finding according to the provisional scoring system for UTx rejection).[10]

Category 2: More than 20 scattered cells containing sparse lymphocytes without clustering within the biopsy sample (corresponding to the normal finding according to the provisional scoring system for UTx rejection).[10] However, these two categories were established for classification purposes, as healthy ectocervical tissues were examined assuming slight inflammatory changes only.

Category 3: Clustering cells containing lymphocytes tending to form small clusters at the epithelial-stromal interface (this can be seen in borderline changes according to the provisional scoring system for UTx rejection).[10]

Category 4: Focal infiltrates containing lymphocytes tending to form large nests or even lymphatic follicles with possible participation of other types of inflammatory cells (this can be seen in grade 1 and 2 rejections according to the provisional scoring system for UTx rejection). Both grades share the same degree of inflammation, and grade 2 was recognized if inflammatory cells were accompanied by stromal edema and reduced surface epithelial thickness.[10]

Category 5: Diffuse infiltrates with continuous inflammatory infiltrate within the tissue (this can be seen in grade 3 according to the provisional scoring system for UTx rejection). These changes might be accompanied by epithelial erosions/ulcerations and focal necrosis.[10]

The basic subsets of T-lymphocytes were evaluated using immunohistochemical markers CD4 and CD8 to identify the dominant types of inflammatory infiltrates and assess similarities to and differences from rejection in the transplanted organ. Both cytotoxic and helper T-cells are T-cell subsets; therefore, a semiquantitative system for the evaluation of the entire T-cell population (using CD45R0) was not used to evaluate these subsets. The subgroup of patients with more than 80% $CD4^+$ or $CD8^+$ lymphocytes was recorded. In the subgroups with lymphocytic infiltration without the prevalence of any subtype or with sparse lymphocytic infiltrate, an admixture of these cells was recorded. Cases with isolated or no T-cells were also recorded.

2.4 | Ethical approval

The study protocol was approved by the Institutional Review Board and Ethics Committee of Motol University Hospital (EK-34/20) on 29 January 2020.

3 | RESULTS

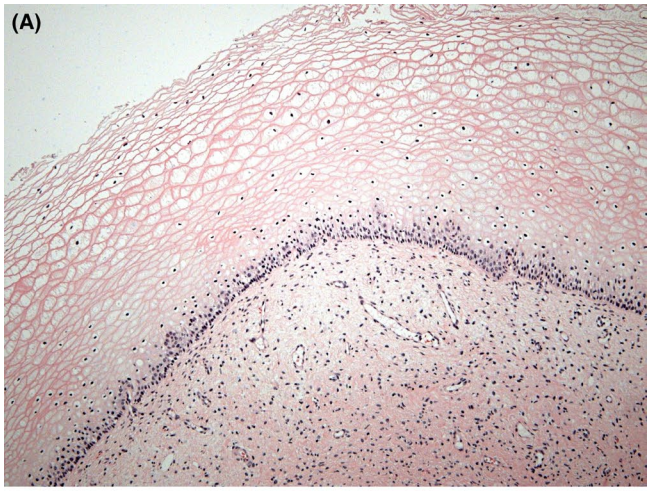
Among the patients in the study group, the mean age was 45.1 ± 3.97 years (range 36–54 years), mean body mass index was 26.6 ± 6.1 (range 17.1–40.8) and the mean parity was 1.9 ± 0.89 (range 0–4). Of the 54 total biopsies, 26 were taken in the proliferation phase vs 28 in the secretion phase of the menstrual cycle; the phases were confirmed by histopathological dating of the endometrium in the removed uteri. A subgroup comparison did not confirm the impact of menstrual cycle phase on histopathological findings in terms of cellular infiltrates in the epithelial–stromal interface and the cervical stroma.

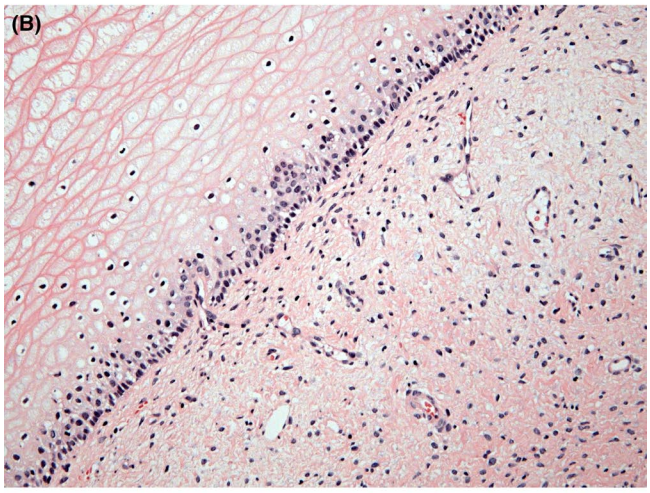
3.1 | Ectocervical results

Evaluation of the biopsies revealed that 52 of 54 samples were suitable for assessment and contained some degree of lymphocytic infiltrate within the ectocervical portion of the uterine cervix; however, none correlated to the first category of the proposed semiquantitative scoring system (Table 1). Similar to other study findings,[10,11] immunohistochemical analysis of the ectocervical lymphocytic population proved that the vast majority of inflammatory cells were T-lymphocytes (positive for immunohistochemical marker CD45RO in all cases). In five (9.6%) samples, lymphocytes were scattered and corresponded to the second category (Figure 2). However, the majority of cases fulfilled the criteria for the third category: 43 (82.7%) showed clustered lymphocytes, and in every sample, inflammatory changes were identified at the epithelial–stromal interface with the intraepithelial influx of isolated lymphocytes (Figure 3). Another four cases (7.7%) contained focal lymphocytic infiltrates (fourth category) (Figure 4), whereas tissue sampling was inadequate in the remaining two participants, from whom only endocervical tissue was obtained. Additionally, no ectocervical samples showed diffuse lymphocytic infiltration correlating with the fifth category. The epithelial influx of lymphocytes within the ectocervix was recorded in 51 (98%) samples. Moreover, lymphocytes were found in the perivascular areas of patients with focal lymphocytic infiltrates (Figure 4). These signs

TABLE 1 Ectocervical biopsy results

	n	%
Samples	52	100
Semiquantitative scoring system of CD45RO-positive T-lymphocytes		
Category 1	0	0
Category 2	5	9.6
Category 3	43	82.7
Category 4	4	7.7
Category 5	0	0
Semiquantitative scoring system of CD^{20+} B-lymphocytes		
Category 1	50	96
Category 2	1	2
Category 3	0	0
Category 4	1	2
Category 5	0	0





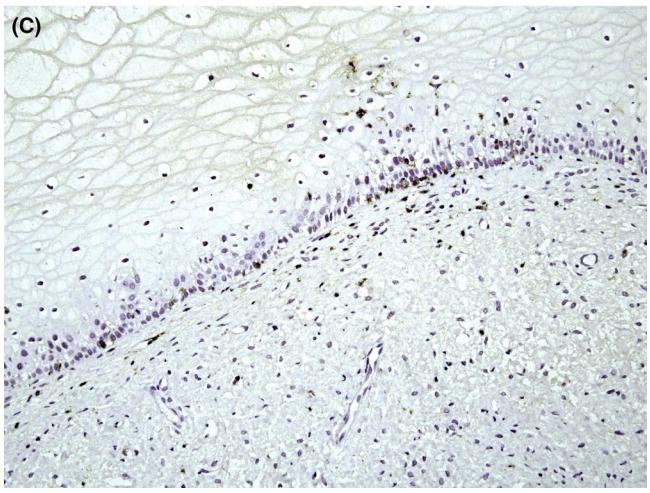


FIGURE 2 The second category of the semiquantitative scoring system proposed by the study. (A) Light microscopy of the ectocervical biopsy (H&E staining). The subepithelial stroma shows sparse inflammatory cells only. This finding is subtle on low magnification. (B) Light microscopy of the ectocervical biopsy (H&E staining). Under higher magnification, single intraepithelial lymphocytes are visible. (C) Light microscopy of the ectocervical biopsy CD45RO staining. The same case with marked T-cells stained using immunohistochemical marker CD45RO. H&E: hematoxylin and eosin

overlap with the borderline change category. None of the cervical biopsies revealed signs of human papilloma virus infection (epithelial dysplasia and/or koilocytosis), endothelialitis or intimal sclerosis of the vessels. These findings are consistent with the histopathological results of the removed uteri.

The T-cells were subdivided into subtypes using the immunohistochemical markers CD8 and CD4. In 24 (46%) cases, $CD8^+$ cytotoxic T-lymphocytes constituted the major cellular fractions. In six (11.5%) cases, T-lymphocytes were scattered and represented an admixture of the inflammatory infiltrate; in 17 (33%) cases, only isolated cells were observed. Five (9.5%) cases were completely negative for the immunohistochemical marker CD8. Two samples contained only endocervical tissue and were inadequate for assessment. There were no (in 49 cases) or only isolated (in three cases) $CD4^+$ helper T-lymphocytes among the lymphocytic population.

Compared with T-cells, B-lymphocytes represented a minority of the total immune cells within the ectocervix, being absent in 41 (79%) cases or isolated in nine (17%) cases (first category), scattered in one (2%) case (second category), and forming focal infiltrates in one (2%) case (fourth category). This finding was consistent with that of a previously suggested provisional scoring system for UTx rejection.[10] The majority of the ectocervical biopsies showed clustered lymphocytic infiltrates affecting the stromal–epithelial interface with epithelial influx, consisting mainly of CD45RO-positive activated T-cells with CD8 T-lymphocyte predominance. $CD4^+$ T-lymphocytes and B-cells were rarely detected. Such immunoprofiles of the immune cell populations shared rejection infiltrates in other transplanted solid organs, such as the lungs.[12,13]

3.2 | Endocervical results

The aim of the biopsy was to take a sample of the ectocervical tissue only, but in 15 (27.8%) cases, a sample of the endocervix was also obtained; all showed some degree of inflammation (Table 2). Compared with the ectocervix, endocervical inflammatory infiltrates were usually larger and recognizable even at low magnification (Figure 5). They were composed of CD45RO-positive T-cells; in 11 of 15 cases, focal infiltrates were noted, representative of the fourth category according to the proposed semiquantitative scoring system. Another three cases contained diffused inflammatory infiltrations (fifth category), whereas the remaining sample showed clustered cells (third category).

The vast majority of the endocervical T-cells were $CD8^+$ T-lymphocytes, representing the main subpopulation of T-cells in 11 cases similar to the ectocervix, but three cases contained isolated Tc lymphocytes and one case was negative. No $CD4^+$ helper cells were identified in the endocervical tissues.

A significant difference was observed in the immunophenotype of the endocervical lymphocytic population vs the ectocervical

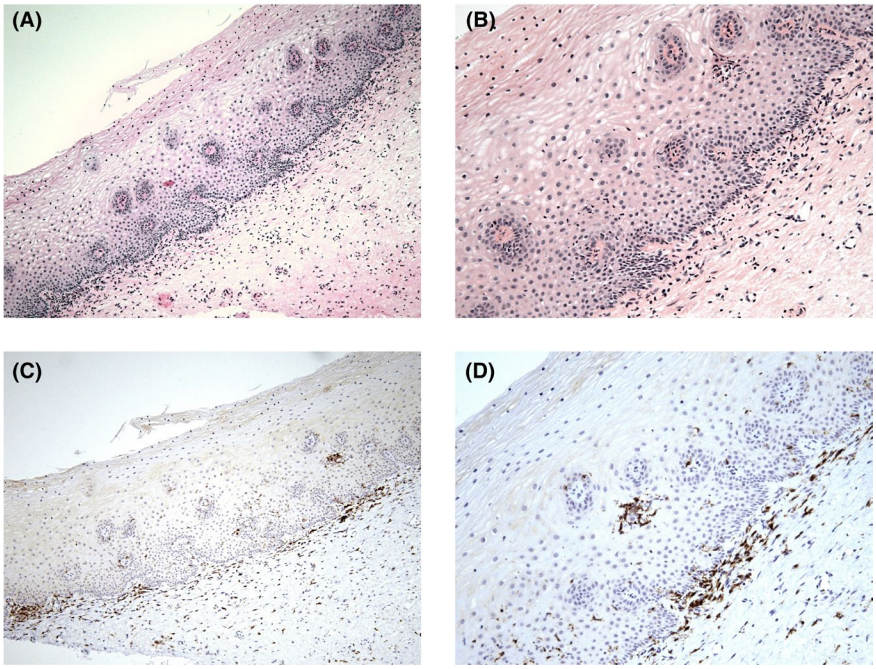


FIGURE 3 The third category of the semiquantitative scoring system proposed by the study. (A) Light microscopy of the ectocervical biopsy (H&E staining). Clustered lymphocytes at the epithelial–stromal junction of the ectocervix appreciated even on low magnification. (B) Light microscopy of the ectocervical biopsy (H&E staining). Details of the intraepithelial influx of clustered lymphocytes found in the majority of cases even with routine H&E staining. (C) Light microscopy of the ectocervical biopsy (CD45RO staining). Immunohistochemical detection of activated T-lymphocytes using CD45RO staining. (D) Light microscopy of the ectocervical biopsy (CD45RO staining). Details of the intraepithelial T-cells. H&E: hematoxylin and eosin

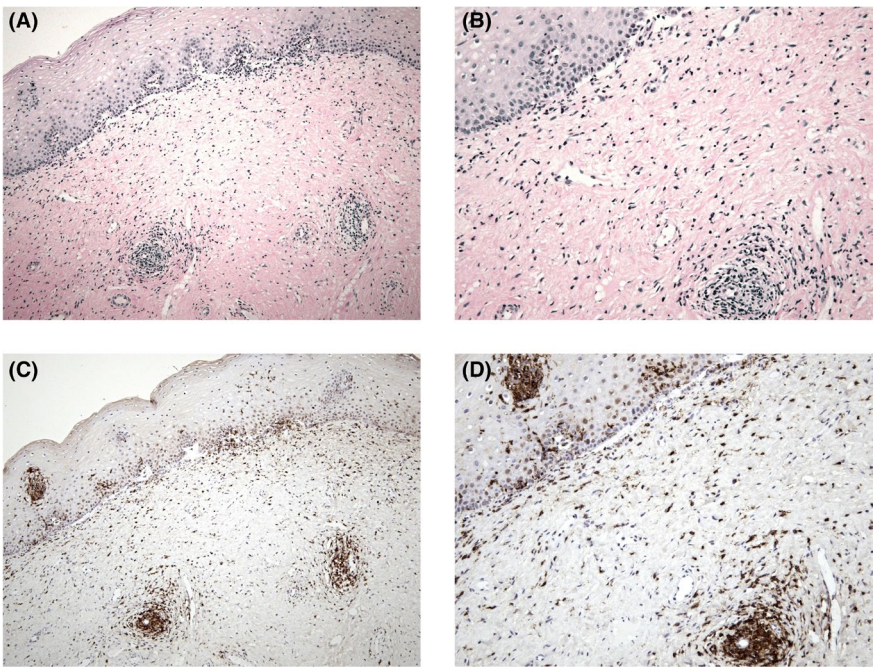


FIGURE 4 The fourth category of the semiquantitative scoring system proposed by the study. (A) Light microscopy of the ectocervical biopsy (H&E staining). Focal lymphocytic infiltrates within the subepithelial stroma. (B) Light microscopy of ectocervical biopsy (H&E staining). Perivascular focal lymphocytic infiltrate and the intraepithelial influx of inflammatory cells. (C) Light microscopy of the ectocervical biopsy (CD45RO staining). The same case is stained with immunohistochemical marker CD45RO. Note the striking intraepithelial influx of lymphocytes. (D) Light microscopy of ectocervical biopsy (CD45RO staining). Details of the previous section. H&E: hematoxylin and eosin

population: A large cohort of endocervical B-lymphocytes stained positive for immunohistochemical marker CD20. The endocervical B-cells were clustered in four of 15 cases, forming focal infiltrates in another 10 and being isolated in one case only. C4d staining was negative, showing weak non-specific positivity in all samples.

4 | DISCUSSION

Here we report the histopathological results of ectocervical biopsies in a group of premenopausal women in relation to rejection changes in the transplanted uterus according to Mölne et al.[10] Findings similar to the borderline changes were noted in 90.4% of our ectocervical

biopsies. This study showed that the histopathological and immunohistochemical findings of ectocervical biopsies from the uteri of premenopausal women without immunity-altering therapy were similar to the borderline category of the provisional scoring system of subclinical graft rejection after UTx.

TABLE 2 Endocervical biopsy results

	n	%
Samples	15	100
Semiquantitative scoring system of CD45RO-positive T-lymphocytes		
Category 1	0	0
Category 2	0	0
Category 3	1	6.7
Category 4	11	73.3
Category 5	3	20
Semiquantitative scoring system of CD^{20+} B-lymphocytes		
Category 1	1	6.7
Category 2	0	0
Category 3	4	26.6
Category 4	10	66.7
Category 5	0	0

To our knowledge, no similar data on the histopathological and immunohistochemical assessments of ectocervical biopsies were published previously. However, in a recently reported study of seven uterine explants, in one of six women of a control group, an ectocervical "focal inflammation with borderline pattern" corresponding to our findings was recorded.[5] Although our study of 54 women showed similar histopathological and immunohistochemical ectocervical findings to the borderline category of uterine rejection used in the ongoing UTx studies, further research in larger groups of non-transplanted premenopausal women are required to validate our findings.

To date, the control of post-transplant uterine rejection using a histopathological examination of the ectocervical biopsy is performed regularly before conception and several times during pregnancy.[1,3,6,8,14] When borderline changes are histopathologically confirmed, an ectocervical re-biopsy is usually conducted. A repeated biopsy can be followed by prolongation of the interval to embryo transfer to allow healing of the post-biopsy epithelial–stromal defects (the cervical biopsy should be taken from the ectocervix close to the cervical canal as suggested by Mölne et al.[10]).

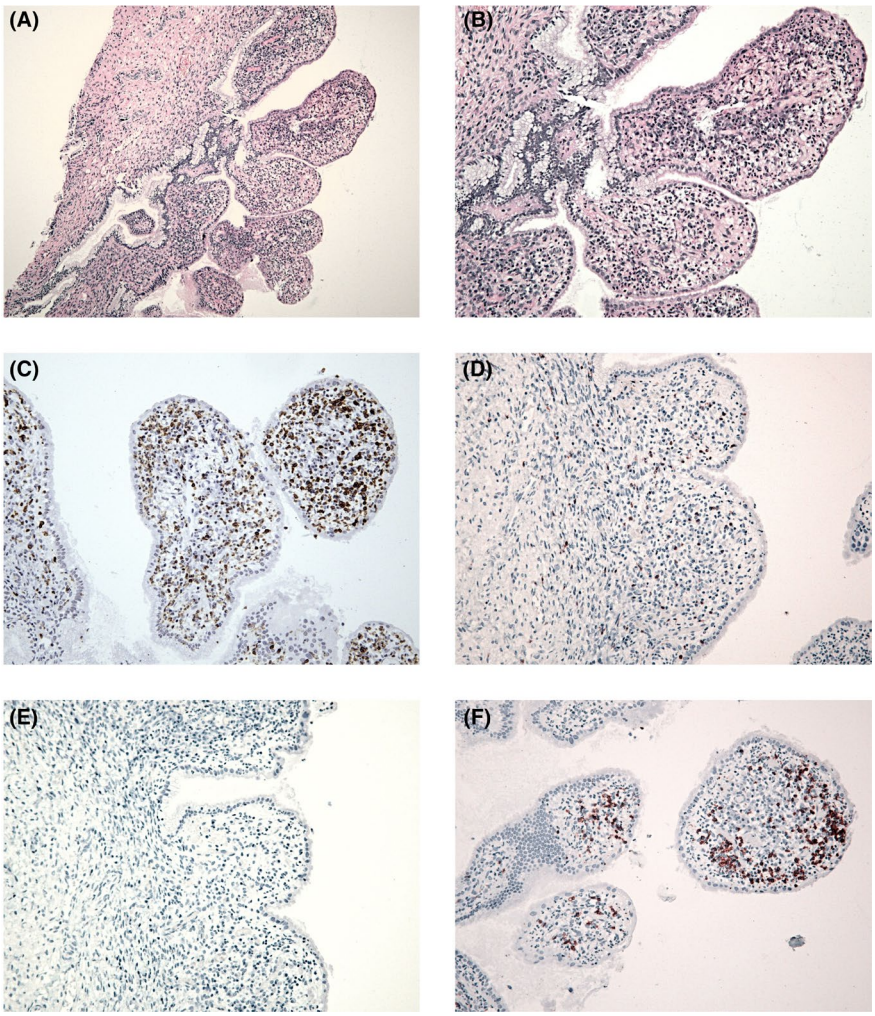


FIGURE 5 Endocervical inflammatory infiltrates. (A) Light microscopy of the endocervical biopsy (H&E staining). Inadequate biopsy sample showing only endocervical tissue. Diffuse inflammatory changes are visible even at low magnification (corresponding to the fifth category of the semiquantitative scoring system). (B) Light microscopy of the endocervical biopsy (H&E staining). Details of the lymphocytic infiltrate within the endocervical stroma. (C) Light microscopy of the endocervical biopsy (CD45RO staining). T-cells are the main lymphocytic population within the endocervix and ectocervix, sometimes forming diffuse infiltrates. (D) Light microscopy of the endocervical biopsy (CD8 staining). $CD8^+$ Tc cells in a population of T-lymphocytes. (E) Light microscopy of the endocervical biopsy (CD4 staining). CD4 staining was negative. (F) Light microscopy of the endocervical biopsy (CD20 staining). Unlike in the ectocervix, there are numerous endocervical B-cells. H&E: hematoxylin and eosin

Posttransplant cervical biopsies should also be performed strictly according to the study protocols regardless of embryo transfer timing.[15] However, although the biopsy itself is not painful, because of the interrupted sensory innervation of the transplanted uterus, frequently performed biopsies may increase the morbidity and discomfort of uterine recipients, which could make its usefulness questionable, particularly in experimental trials testing the efficacy and safety of new methods.

The grade 0 category of the organ transplantation rejection scale was established to reveal an imminent rejection of an organ that is either vital (such as the kidney or lungs) or to improve quality of life (such as the hand or face). Based on current knowledge of UTx research, it is highly speculative to claim that borderline changes in the ectocervical biopsy after UTx are normal, although our study results suggest this. We agree with the recent proposal to keep the category of "inflammation of uncertain significance" (borderline category), although a similar inflammatory pattern has been observed in non-transplanted ectocervical tissue as well as in women with uterine transplants and rejection signs at other points.[5]

We believe that using the optimal technique for cervical biopsy is crucial to ensuring an accurate assessment of cervical samples. Our data showed differences between lymphocytic infiltration within the ectocervical and endocervical tissues. The endocervical interstitium contained lymphocytes that correlated with higher scores on the proposed semiquantitative scoring system. The ectocervical biopsy can also contain areas of endocervical tissue; however, when only an endocervical stromal component is obtained, this may be confused with rejection. Therefore, a cervical biopsy after UTx should be performed by an experienced gynecologist using colposcopy magnification, which aids the identification of the optimal part of the ectocervix to biopsy to minimize the risk of incorrect sampling, for example, from a scar after a previous cervical biopsy. However, when only the endocervical stroma and no characteristic endocervical epithelial structures are detected, immunohistochemistry of the lymphocytes can identify the tissue's origin because numerous $CD20^+$ B-lymphocytes are detected in the endocervix but rarely in the ectocervix.

Until the discovery of less invasive methods of rejection control, ectocervical biopsy was the only safe method for the early detection of rejection signs after UTx. The pioneering experience of the first Swedish UTx trial was based solely on signs of cellular histopathological rejection; subsequent UTx studies confirmed these findings.[1,8] The only different case report on uterine rejection after transplantation citing the first-ever severe mixed cell/humoral rejection, which was reversed by multiple thymoglobulin administrations, was recently published.[16] However, only further data can confirm the above experience with the humoral component of rejection in uterine transplants.

This study had several strengths. First, it included a large number of participants. Second, it had a prospective design. Third, it suggested a semiquantitative scoring system to enhance its reproducibility. Finally, the ectocervical biopsies were taken from a group of women undergoing hysterectomy under general anesthesia and did not impact their morbidity.

This study also had some limitations. First, to the best of our knowledge, no previous reports are available for comparison. Second, this was only a single-center study. Third, our data lacked statistical support.

5 | CONCLUSION

To our knowledge, this prospective non-randomized study was the first to assess the histopathological and immunohistochemical findings of ectocervical biopsies in premenopausal women in relation to the provisional scoring system of rejection changes in transplanted uteri. Our study showed similar histopathological findings to the borderline category of the above scoring system of rejection used in previous UTx studies. However, similar studies are required to validate our findings.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

JB: data collection, data interpretation, manuscript writing. MN and RC: study design, data collection, data interpretation, manuscript writing. PS, ZP and RCJr: study design, data interpretation, manuscript writing. JZ: data interpretation, manuscript writing.

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Mapping of the lung megakaryocytes: A role in pathogenesis of idiopathic pulmonary arterial hypertension?

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ABSTRACT

It has been postulated that platelets are produced by fragmentation of the megakaryocytes within the pulmonary circulation rather than budding of their cytoplasm within the bone marrow. Although literature is scarce depicting the levels of the megakaryocytes within the lungs from previously healthy individuals, there are several studies describing the presence of these cells in human necropsy specimens, and it has been hypothesized that their rearrangements could contribute to the pathogenesis of chronic pulmonary vascular disorders. The objective of this study was to describe the characteristics, distribution and total count of megakaryocytes in explants from lung transplant (LTx) recipients based on the final clinicopathological diagnosis, as well as in samples from LTx donors without previously known pulmonary disease. Using the immunohistochemical marker CD61 we quantified and characterized such cells in 20 biopsy samples from LTx donors and in 30 biopsy samples from LTx recipients with different pathologic conditions: vascular disorders of the lungs, obstructive pulmonary disorders and fibrotic lung diseases. Patients suffering from idiopathic pulmonary arterial hypertension (IPAH) showed morphological differences and strikingly higher numbers of the lungs megakaryocytes (264.5 cells/cm^2) compared to all the other groups (the average count among donors was 33.55 megakaryocytes/cm^2). Such finding could contribute to the understanding of the origin of vasoconstriction, thrombosis and vascular remodeling of the pulmonary circulation – all the basic mechanisms leading to the development of IPAH, as for there is an increasing evidence of several products of platelets and megakaryocytes to be capable of triggering such processes.

1. Introduction

It was postulated a long time ago that platelets are produced by fragmentation of the megakaryocytes within the pulmonary circulation rather than budding of their cytoplasm within the bone marrow. The contribution of the lungs to platelet biogenesis is substantial, accounting for approximately 50% of total platelet production [1–5]. Among others, the thesis of platelets production within the lungs was proven by comparing the difference in intact megakaryocytes count between pulmonary and aortic arterial blood [4] as well as pulmonary arterial and venous blood [6]. There are also several studies describing the presence of megakaryocytes within healthy lung tissue in mouse models [1,7].

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However, there are only isolated studies among human lung tissue, which partially specify the occurrence of the lung megakaryocytes within cadaverous lungs based on necropsy specimens [8,9]. These studies establish the mean count of the megakaryocytes within normal lungs to be 14.65 cells/cm^2 [8] and 16.8 cells/cm^2, respectively [9].

Nevertheless, the autopsy samples share the risk of autolysis, which can misrepresent the true cell count, especially in populations of large fragile cells such as megakaryocytes. On the other hand, it is hard to obtain representatively large sized samples of healthy lung tissue (1 cm^2) in living patients by standard transbronchial biopsies or even cryobiopsies.

The objective of this study was to describe the characteristics, distribution and total count of megakaryocytes in explants from lung transplant recipients based on the final clinicopathological diagnosis, as well as in samples from LTx donors without previously known pulmonary disease. The lung tissue obtained from the lung transplantation (LuTx) program was used to reveal more accurate physiological count of the megakaryocytes within lungs, unaffected by post mortem autolysis. We examined either unused donor lungs (if single LuTx was performed) or parenchyma resected to decrease the volume of the oversized graft during double LuTx (usually the lingula of the left lung or the middle lobe of the right). Therefore, even though all the donors represented cadaverous individuals, such samples provide more accurate information regarding cell populations because of the avoidance of autolytic changes due to the application of preservation solution and assuring of short time of cold ischemia prior to the LuTx, followed by the immediate formalin fixation after the resection. Except from the donor lungs without any previous pulmonary disease, this study targets examination of lung megakaryocytes within pathologically changed lung explants obtained from recipients, as there is an increasing evidence of interconnection of abnormal megakaryocytes functions and vascular diseases [3,10,11].

2. Materials and methods

The study cohort consisted of 50 individuals included in the LuTx program between 2015 and 2021 at Motol University Hospital in Prague, Czech Republic, based on their indication diagnoses (recipients) or accessibility of the unused lung parenchyma (donors). Informed consent was obtained from each participant for experimentation with surgically removed organs and tissues. For the mapping of the donor lungs, the oversized or single LuTx lung explants from human donors (n = 20) were used. The lung parenchyma was macroscopically examined and considered suitable for LuTx purposes before the harvest itself. Subsequently, the absence of any pulmonary disease was proven also histologically in all grafts.

For the mapping of the lung megakaryocytes among pathologically changed lungs, the explants from recipients (n = 30) were examined and divided into 3 cohorts based on the character of their original condition – vascular (n = 10), restrictive (n = 10) and obstructive (n = 10) lung diseases.

2.1. The following groups were formed

a) Cadaverous donors (n = 20) from whom the lung parenchyma was harvested prior to LuTx before the onset of autolysis and in which the further histological examination did not prove any pulmonary disease (male/female: 6/14, age: median 55 y, range 30–76 y). All the harvested lungs were immediately kept in the preservation solution (Perfadex) and the time of cold ischemia did not exceed 8 h. During the LuTx itself, the material from oversized lungs and single LuTx was fixed in formalin immediately after the resection. For more details on donor characteristics see Table 1.
b) Recipients with vascular pulmonary disorders (n = 10): This group included lung explants of 8 patients with idiopathic pulmonary arterial hypertension (IPAH), 1 patient with veno-occlusive disease and 1 patient with pulmonary capillary hemangiomatosis. (male/female: 1/9, age: median 26.7 y, range: 18–36 y).
c) Recipients with restrictive pulmonary disease (n = 10): Lung explants of 6 patients suffering from chronic hypersensitivity pneumonitis (CHP), 2 cases of pleuroparenchymal fibroelastosis (PPFE), 1 patient with idiopathic pulmonary fibrosis (IPF) and 1 with rheumatoid lung disease (male/female: 6/4, age: median 50.9 y, range: 45–61 y).
d) Recipients with obstructive pulmonary disease (n = 10): Lung explants of 9 patients with chronic obstructive pulmonary disease (COPD) and 1 case with alpha-1-antitrypsin deficiency (AATD) (male/female: 6/4, age: median 60.4 y, range: 56–66 y).

The clinical follow-up, CT scans and biopsy evaluations were correlated to conclude final diagnosis in all cases. Equal distribution of left and right lung specimens was ensured. Furthermore none of the patients

Table 1
patient characteristics of donors and their pulmonary megakaryocytes.

Patient	Sex	Age (y.)	Cause of death	Type of the lung explant	Megakaryocyes/ cm^2	Localization of the megakaryocytes	The largest size of the megakaryocytes
1	M	46	SAH	LL	6	interalveolar septa	small (isolated)
2	M	56	CVA	RML	47	interalveolar septa	medium (isolated)
3	M	42	CE	RML	36	interalveolar septa	medium (isolated)
4	F	60	CVA	L	22	interalveolar septa	medium (isolated)
5	F	71	CE	RML	31	interalveolar septa	medium (isolated)
6	M	39	CE	RML	76	interalveolar septa	large (isolated)
7	F	76	SAH	RML	33	interalveolar septa	medium (isolated)
8	F	67	SAH	RL	17	interalveolar septa	medium (isolated)
9	F	69	SAH	RML	28	interalveolar septa	medium (isolated)
10	F	54	CAR	RML	83	interalveolar septa	large (isolated)
11	F	50	SAH	RML	28	interalveolar septa	medium (isolated)
12	F	71	CVA	RUL	9	interalveolar septa	medium (isolated)
13	F	35	CE	L	44	interalveolar septa	medium (isolated)
14	F	60	SAH	RML	15	interalveolar septa	small (isolated)
15	M	53	CVA	L	25	interalveolar septa	medium (isolated)
16	F	46	CVA	L	18	interalveolar septa	medium (isolated)
17	F	64	SAH	LL	16	interalveolar septa	medium (isolated)
18	F	54	SAH	RML	36	interalveolar septa	small (isolated)
19	F	62	CVA	L	21	interalveolar septa	medium (isolated)
20	M	30	TBI	RML	80	interalveolar septa	medium (isolated)

Abbreviations: y, years; M, male; F, female; SAH, subarachnoid hemorrhage; CVA, cerebrovascular accident; CE, cerebral edema; CAR, carotid artery rupture; TBI, traumatic brain injury; LL, left lung; RL, right lung; RML, right middle lobe; RUL, right upper lobe; L, lingula.

included in the study suffered from myeloproliferative disease or any other malignancy, which could distort the levels of megakaryocytes and would be considered as a contraindication for transplantation itself. For more details on recipient characteristics, see Table 2.

2.2. Histopathology and immunohistochemistry

All the lung explants were fixed in neutral buffered 4% formaldehyde, transported to a histopathological laboratory and postfixed. Based on macroscopy finding, samples were taken from each explant and embedded in 1 cm^3 sized paraffine blocks. The paraffin blocks were sectioned into 4-μm-thin histological sections and stained with hematoxylin-eosin. For immunohistochemistry, 3-μm-thin histological sections were used, and every biopsy sample was stained using anti-CD61 mouse monoclonal antibodies (clone 2f2, BioSB, dilution 1:100; pre-treatment: heating up to 99 °C in a pH 9 buffer in a water bath). Detection was performed using a one-step micropolymeric non-Biotin system (BioSB, Santa Barbara, CA, USA) with a peroxidase complex and DAB (3, 3 ́-diaminobenzidine tetra-hydrochloride). The nuclei were counterstained with hematoxylin. The paraffine blocks from the lungs of recipients were chosen randomly to avoid any distortion caused by selective sampling of pathologically changed/unchanged lung parenchyma. Only the equal distribution of left and right lung specimens was ensured.

Lung megakaryocytes were identified as CD61 positive cells with visible nuclei to determine them from accumulated platelets/platelet thrombi. The absolute count of such cells was quantified per 1 cm^2 of lung tissue using a grid labeled on the histology slide. The results of every recipient group were compared with those of the donors and the differences were statistically validated (p value was calculated using t-test). For characterization of megakaryocytes, their localization and size was noted, using comparison to erythrocytes as universal histological scale (uniform diameter of the erythrocyte is 7.5 μm), dividing megakaryocytes into large ones (defined by diameter of 50–100 μm; showing typical image of bone marrow-localized megakaryocytes with abundant cytoplasm), medium sized ones (20–49 μm; with moderate amount of cytoplasm) and small forms (< 20 μm; with a thin rim of cytoplasm). The levels of blood platelets of the recipients (examined 1 day prior to LuTx) were noted too for every case.

Table 2
patient characteristics of recipients and their pulmonary megakaryocytes.

Patient	Sex	Age (y.)	Diagnosis	Mean PH (mm/Hg)		Type of the lung explant	Platelets x10^9/l (1 day prior LuTx)	Megakaryocyes/ cm^2	Localization of the megakaryocytes	The largest size of the megakaryocytes
Recipients with vascular pulmonary disease:										
1	F	28	IPAH	55		LL	95	342	IS	large (CL)
2	F	18	IPAH	74		RL	80	322	IS	large (CL)
3	F	28	IPAH	55		RL	208	148	IS	medium (CL)
4	F	21	VOD	42		RL	93	101	IS	medium (CL)
5	F	32	IPAH	67		LL	159	764	IS	large (CL)
6	F	36	PCH	78		LL	160	33	IS	medium (CL)
7	M	32	IPAH	38		RL	265	62	IS	large (CL)
8	F	19	IPAH	69		LL	99	100	IS	large (CL)
9	F	21	IPAH	76		LL	179	247	IS	large (CL)
10	F	32	IPAH	59		LL	79	131	IS	large (CL)
Recipients with restrictive pulmonary disease:										
Patient	**Sex**	**Age (y.)**	**Diagnosis**	**Mean PH (mm/Hg)**	**FVC (%)**	**Type of the lung explant**	**Platelets x10^9/l (1 day prior LuTx)**	**Megakaryocyes/ cm^2**	**Localization of the megakaryocytes**	**The largest size of the megakaryocytes**
1	M	50	CHP	11	55.1	RL	183	6	IS	medium (I)
2	M	51	CHP	44	47.3	LL	82	9	IS	medium (I)
3	M	53	RLD	20	28.9	RL	230	3	IS	medium (I)
4	M	46	CHP	19	32.7	LL	136	15	IS	medium (I)
5	M	49	CHP	16	48.4	RL	282	11	IS	medium (I)
6	F	61	CHP	26	31.1	LL	229	2	IS	medium (I)
7	M	55	IPF	17	55.7	RR	249	2	IS	medium (I)
8	F	49	CHP	37	26.6	RR	320	6	IS	medium (I)
9	F	50	PPFE	20	29.3	LL	248	3	IS	medium (I)
10	F	45	PPFE	23	17.5	LL	135	1	IS	small (I)
Recipients with obstructive pulmonary disease:										
Patient	**Sex**	**Age (y.)**	**Diagnosis**	**Mean PH (mm/Hg)**	**FEV1 (%)**	**Type of the lung explant**	**Platelets x10^9/l (1 day prior LuTx)**	**Megakaryocyes/ cm^2**	**Localization of the megakaryocytes**	**The largest size of the megakaryocytes**
1	F	57	COPD	21	17.1	RL	114	17	IS	medium (I)
2	M	65	COPD	27	18.9	LL	240	30	IS	medium (I)
3	M	59	COPD	24	30.5	LL	154	2	IS	medium (I)
4	M	56	COPD	35	19.0	RL	354	29	IS	large (I)
5	M	66	COPD	27	23.2	LL	267	8	IS	small (I)
6	F	57	COPD	31	21.4	RL	158	4	IS	medium (I)
7	M	63	COPD	38	34.1	LL	424	12	IS	medium (I)
8	F	59	AATD	13	35.1	RL	186	6	IS	medium (I)
9	F	64	COPD	17	23.9	RL	270	3	IS	medium (I)
10	M	58	COPD	36	22.5	LL	258	8	IS	small (I)

Abbreviations: y, years; M, male; F, female; LL, left lung; RL, right lung; LuTx, lung transplantation; IS, interalveolar septa; I, isolated; CL, clustering; PH, pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension; VOD, veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis; CHP, chronic hypersensitivity pneumonitis; RLD, rheumatoid lung disease; IPF, idiopathic pulmonary fibrosis; PPFE, pleuroparenchymal fibroelastosis; COPD, chronic obstructive pulmonary disease; AATD, alpha-1 antitrypsin deficiency.

3. Results

Among donors (n = 20), the megakaryocytes showed interindividual variability. Their mean count±SD was 33.55 ± 22,56megakaryocytes/cm^2 (range: 6–83 megakaryocytes/cm^2). In all cases, the megakaryocytes were localized within the capillary lumen of the interalveolar septa of the lung. Their cytomorphology represented mainly medium forms in 15 cases (75%). 3 cases (15%) contained small forms of the lung megakaryocytes with a thin rim of cytoplasm only, resembling naked or semi naked nuclei. The remaining large forms possessing abundant cytoplasm were found in 2 patients (10 %). All megakaryocytes were isolated without any tendency to form clusters or aggregates (see Fig. 1). For more details on the count of megakaryocytes of the donors, see Table 1 and Fig. 2.

Among recipients (n = 30), the average number of the lung megakaryocytes differed greatly according to the character of the baseline disease. The largest density of these cells was observed in the group of patients suffering from vascular pulmonary disorders (n = 10), being mean±SD: 225 ± 216.87megakaryocytes/cm^2 (range: 33–764 megakaryocytes/cm^2). This finding represents statistically valid difference compared to donors (p value = 0.02105). Moreover, there was 1 patient with pulmonary capillary hemangiomatosis in this cohort, who showed a very low level of megakaryocytes (33 cells/cm^2) and 1 case of veno-occlusive disease of the lungs, also with lower megakaryocytic count (101 megakaryocytes/cm^2). All the remaining patients suffered from IPAH. If the patients with IPAH are considered only, the megakaryocytic count increased as follows: mean±SD: 264.5 ± 226.33 megakaryocytes/cm^2 (range: 62–764 megakaryocytes/cm^2). The group of patients with vascular pulmonary disease was also the only one showing certain morphological features, such as clustering of the megakaryocytes – there were agglomerates of the megakaryocytes within capillaries of the interalveolar septa detected in each case (see Fig. 3). Different cytomorphology of the lung megakaryocytes was also noted among this group – in 6 cases (60 %) there were large forms of such cells visible. The remaining 4 cases (40%) contained medium sized megakaryocytes.

The lowest density of the lung megakaryocytes was observed among patients suffering from a restrictive pulmonary diseases (n = 10) with mean±SD: 5.8 ± 4.59megakaryocytes/cm^2 (range: 1–15 megakaryocytes/cm^2). This finding represents statistically valid difference compared to donors (p value < 0.0001). As in the donor lungs, the megakaryocytes were isolated and placed inside capillaries of interalveolar septa in all cases, none case showed their clustering. They represented mainly medium forms (90%) with 1 exception containing small sized cells only (10 %).

Compared to the donor lungs without pulmonary diseases, the mean megakaryocytic density was also lower among the group of patients with obstructive pulmonary diseases (n = 10), but not in such severe extent as the restrictive ones: mean±SD: 11.9 ± 10.28 megakaryocytes/cm^2 (range: 2–30 megakaryocytes/cm^2). This finding represents statistically valid difference compared to donors (p value = 0.001194). The lung megakaryocytes were isolated and localized within capillaries of the interalveolar septa in all cases. In 7 cases (70%) they showed medium sized forms and in the remaining 3 cases, one large and two small forms were observed.

For more details on the count of megakaryocytes of the recipients, see Table 2 and Fig. 2.

There was no correlation between the density of the lung megakaryocytes and blood levels of platelets of the recipients (examined 1 day prior to LuTx). The blood levels of platelets of the donors were not available prior the harvest.

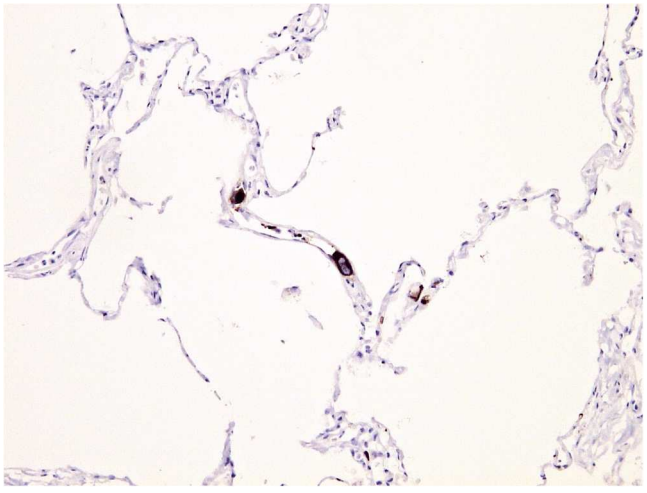

Fig. 1. The lung megakaryocytes within healthy lung tissue of the donor. Light microscopy of the lung (CD61 staining; objective: magnification 20x). Immunohistochemical detection of the isolated and medium sized megakaryocytes, localized within the lumen of the capillaries of the interalveolar septa.

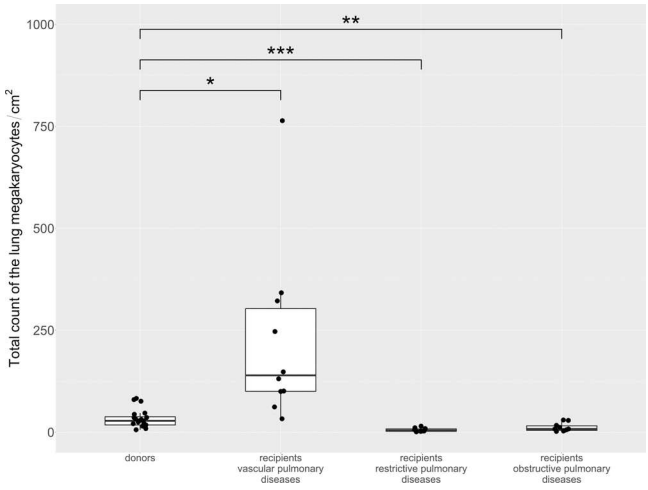

Fig. 2. Boxplots with overlayed dots showing statistics and specific counts of megakaryocytes per square centimeter in individual groups of patients and controls. * - p < 0.05, ** - p < 0.01, *** - p < 0.001.

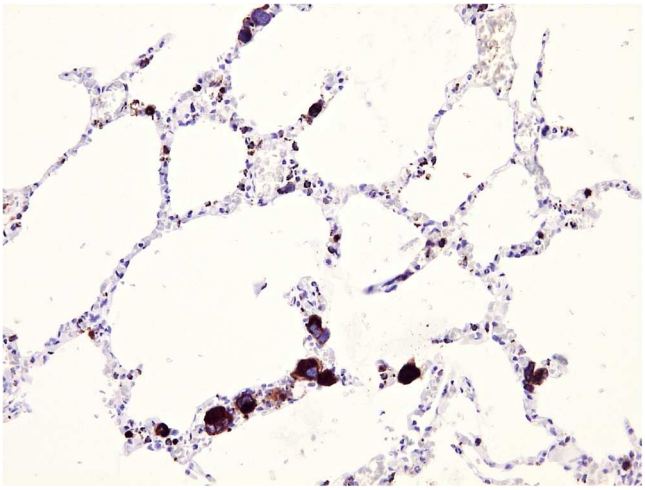

Fig. 3. The lung megakaryocytes within lung tissue of the recipient suffering from IPAH. Light microscopy of the lung (CD61 staining; objective: magnification 20x). Immunohistochemical detection of the clustering and large megakaryocytes, localized within the lumen of the capillaries of the interalveolar septa.

4. Discussion

This study focused on mapping of the pulmonary megakaryocytes among LuTx donor lungs without any previous pulmonary disease and LuTx recipients suffering from different pulmonary disorders. These graft specimens promise accurate representation of the megakaryocytic levels as for donor lung are intact and protected from autolysis compared to the necropsy samples from the previous studies [8,9]. The samples from LuTx recipients were also protected from autolysis due to the immediate formalin fixation of the bioptic material after the explantation.

There was an interindividual variability in the population of the lung megakaryocytes. However, tendency for different average count among every cohort was observed with statistically proven significant differences between donors and recipients of every group. (For more details see Fig. 2.) The mean count of the pulmonary megakaryocytes in donor lungs was slightly higher (33.55 cells/cm^2), compared to the previous studies [8,9], perhaps because of the avoidance of autolytic changes. Meanwhile the mean count among recipient lungs affected with the vascular pulmonary disorders reached 225 megakaryocytes/cm^2, striking even 264.5 megakaryocytes/cm^2 if the patients with IPAH were considered alone. This finding represents statistically valid difference compared to donors (p value = 0.02105) and all the other groups of recipients, which showed even lower megakaryocytic counts.

The mean count of the lung megakaryocytes was much lower in recipients suffering from restrictive lung diseases (5.8 megakaryocytes/cm^2, p value < 0.0001 in comparison with donors) and slightly higher than this in recipients with obstructive lung diseases (11.9 megakaryocytes/cm^2, p value = 0.001194 in comparison with donors). There was also different distribution pattern of the lung megakaryocytes among the group of the patients with vascular pulmonary disorders – in all cases the clustering of these cells was noted. However, there was no such feature observed in other cohorts. Moreover, the cytomorphology of the lung megakaryocytes among the recipients with vascular pulmonary diseases differed as well. Their megakaryocytes represented mainly large forms (in 60%) - a finding, which was rarely detected in the other groups.

Considering the postulated role of the lung megakaryocytes in platelets formation [1–5], these conspicuous differences in the mean count, distribution and morphology of the lung megakaryocytes could be related to or contribute to the etiopathology of the IPAH as there is an increasing evidence of platelets playing a key role in the development of the pulmonary hypertension [12–14]. Moreover, some recent publications showed that not only platelet derived cytokines can affect remodeling of the pulmonary arteries [15,16], but also several products of the megakaryocytes can promote development of thrombi and angiogenesis too (such as prothrombogenic and proangiogenic factors like von Willebrand factor, vascular endothelial growth factor-A, fibroblast growth factor 2, epidermal growth factor, platelet-derived growth factor and matrix metallopeptidase 9) [12,16–21]. Von Willebrand factor tends to be even elevated in the blood IPAH patients [22]. Perhaps the defect of the budding of the megakaryocyte cytoplasm could explain the presence of large forms in such high numbers within lung tissue of IPAH patients, described in this study. Facilitating the idea of the platelet formation disorder, some publications suggest, there is an accelerated turnover of the platelets in IPAH patients [23]. Similar abnormities in the count, distribution and morphology of the megakaryocytes, including their clustering, can be observed in disorders of the bone marrow (the primary site of megakaryocyte production), such as myelodysplasia and myeloproliferative diseases. However, future data are required to confirm this hypothesis.

The feature impeaching the simple storage of these cells in the lungs affected with IPAH is the presence of the lung megakaryocytes within capillaries of the interalveolar septa, which was observed in all cases in our cohort. The capillaries are localized after the arterial stenosis, therefore intracapillary cells already had to pass the stenotic area and are not just contained within pre-stenotic blood stasis.

The low mean counts of the lung megakaryocytes in terms of the restrictive and obstructive pulmonary diseases could be explained by the loss the lung parenchyma alongside with the destruction of interalveolar septa because of interstitial fibrosis in the first case and because of the air trapping leading to the emphysematous changes in the latter.

This study has some limitations. It analyzed a relatively small sample size and larger cohorts should confirm the acquired data. Furthermore some limitation could be seen in lung harvesting in terms of the perfusion of the donor lungs. However despite the instillation of perfusion medium (Perfadex) into the donor lungs, there was no difference noted in intraluminal corpuscles within vessels compared to the recipient's lungs on histopathology level. Also, the lungs of cadaverous donors cannot be considered as absolutely healthy because the increased permeability of capillaries and neurogenic pulmonary oedema with endothelial damage tends to be developed after the brain death [24].

However, our study has several strengths. To our knowledge, it is the first one to describe the levels of the lung megakaryocytes within lung parenchyma using biopsy material from LuTx program. LuTx explants allow studying these cells within the large tissue area (such as per 1 cm^2) without any added risk or unnecessary procedure for neither donors, nor recipients. In contrast, the previous publications used mouse models [1,7] or necropsy specimens [8,9]. It also depicts conspicuous differences in the mean count of the lung megakaryocytes based on the original pulmonary disease with striking quantitative and qualitative changes of such cells in IPAH patients. Such feature correlates with recent findings of platelets playing a key role in the development of the pulmonary hypertension [12–14].

5. Conclusions

This study focused on mapping of the pulmonary megakaryocytes among LuTx donor lungs without any previous pulmonary disease and LuTx recipients suffering from different pulmonary disorders. Patients suffering from idiopathic pulmonary arterial hypertension (IPAH) showed morphological differences and strikingly higher and statistically validated counts of the lungs megakaryocytes (264.5 cells/cm^2) compared to all the other groups (the average count among donors was 33.55 megakaryocytes/cm^2). Such finding could contribute to the understanding of the origin of vasoconstriction, thrombosis and vascular remodeling of the pulmonary circulation – all the basic mechanisms leading to the development of IPAH, as for there is an increasing evidence of several products of platelets and megakaryocytes to be capable of triggering such processes.

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CRediT authorship contribution statements

Jan Balko: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Jan Havlin:** Data curation, Formal analysis, Writing – review & editing. **Fernando Casas Mendez:** Data curation, Formal analysis, Writing – review & editing. **Andrea Zajacova:** Data curation, Formal analysis, Writing – review & editing. **Miroslav Koblizek:** Data curation, Visualization. **Monika Svorcova:** Writing – review & editing. **Robert Lischke:** Supervision. **Josef Zamecnik:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Transplantace dělohy v léčbě ženské neplodnosti z pohledu patologa

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SOUHRN

Transplantace dělohy představuje novou experimentální metodu léčby absolutního uterinního faktoru infertility, který postihuje 3-5 % neplodných žen. Jedná se zejména o ženy s uterinní agenezí, některými závažnými kongenitálními malformacemi dělohy, získanými chorobami dělohy vedoucími k neplodnosti a stavy po hysterektomii u žen fertilního věku. Umožnění reprodukce biologicky vlastních potomků řadí transplantaci dělohy mezi jednu z možností léčby sterility pomocí metod asistované reprodukce, která však bývá v současnosti některými etiky označována jako příliš radikální forma lidské reprodukce. Analýza stavu novorozenců narozených z transplantované dělohy ukazuje vysokou míru porodů dětí pomocí císařského řezu ve stádiu zralosti nebo lehké nezralosti a nenaznačuje závažnější zdravotní komplikace na straně matek ani dětí. Transplantace dělohy je proto dnes vnímána jako nadějná metoda léčby absolutního uterinního faktoru infertility, která má potenciál doplnit další cesty vedoucí k dosažení mateřství u žen s nefunkční či absentující dělohou, tedy surogátní těhotenství a adopci.

Patolog je podobně jako v případě dalších orgánových transplantací jedním ze základních členů multidisciplinárního týmu. Jeho hlavní role spočívá v hodnocení rejekčních změn uterinních alograftů ve vzorcích z biopsie ektocervixu děložního hrdla, která je dosud jedinou možností kontroly hrozící rejekce transplantované dělohy. Evaluace známek rejekce dělohy vychází z platného tzv. provizorního skórovacího systému navrženého švédskými průkopníky na poli výzkumu transplantace dělohy, který slovem provizorní ve svém názvu naznačuje potřebu dalšího výzkumu této problematiky.

Klíčová slova: transplantace – děloha – rejekce – cervikální biopsie – grading

Uterus transplantation in the treatment of female infertility: the pathologist's perspective

SUMMARY

Uterus transplantation is a new experimental treatment method of absolute uterine factor infertility which affects 3-5% of infertile women. Absolute uterine factor infertility includes infertile women with agenesis or severe malformation of the uterus, several acquired uterine diseases causing infertility, and patients of fertile age after hysterectomy because of various causes. Uterus transplantation is considered a new method of assisted reproduction which allows women with absolute uterine factor infertility to have own biological offspring. However, uterus transplantation is considered a radical method of reproduction by some ethicists. Nevertheless, recent analysis of newborns from transplanted uterus has shown high level of childbirths of mature and near-to-term newborns and did not confirm increased risk for both babies and mothers. Therefore, together with gestational surrogacy and adoption, uterus transplantation is nowadays considered promising and unique solution for women with absolute uterine factor infertility.

Similarly to other solid organ transplants, the pathologist should be an integral part of the multidisciplinary uterus transplantation research teams. The primary role of the pathologist is histopathological evaluation of rejection changes in the biopsy samples from the ectocervix of the uterine allografts that is based on the provisional scoring system suggested by Swedish pioneers in uterus transplantation research. As the word provisional suggests, this scoring system is continuously studied and the principles of the evaluation of rejection after uterus transplantation could be adjusted in the future.

Keywords: transplantation – uterus – rejection – ectocervical biopsy – grading

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Transplantace dělohy (**UTx**) představuje novou experimentální metodu léčby absolutního uterinního faktoru infertility (AUFI), který postihuje 3-5 % neplodných žen. Jedná se zejména o ženy s uterinní agenezí, závažnými malformacemi dělohy, získanými chorobami dělohy ovlivňujícími fertilitu (např. intrauterinní adheze a leiomyomy deformující děložní dutinu) a pacientky ve fertilním věku po hysterektomii (1). UTx neslouží k záchraně života a zdraví (jako např. transplantace srdce, plic, jater či ledvin), ale patří do skupiny tzv. vaskulárně-kompozitních alotransplantací (např. transplantace obličeje a ruky), které jsou určené ke zvýšení kvality života a zkvalitnění sociální integrace jedinců do společnosti. UTx se však jiným orgánovým transplantacím vymyká zejména tím, že jde o jedinou primárně plánovanou dočasnou transplantací a užívání imunosupresivní terapie je proto nutné pouze do doby naplnění reprodukčních cílů příjemkyně. Poté je děložní štěp z těla vyjmut a antirejekční léčba může být ukončena. Možnost reprodukce biologicky vlastních potomků řadí UTx mezi metody asistované reprodukce, ale některými zastánci adopcí bývá označována i jako příliš náročná a relativně radikální forma lidské reprodukce (2).

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HISTORIE TRANSPLANTACE DĚLOHY

První humánní transplantace dělohy proběhla v roce 2000 v Saudské Arábii, kdy příjemkyně dělohy přijala štěp od nepříbuzné žijící dárkyně. Ačkoli samotný odběr i transplantace proběhly úspěšně, po 3 měsících od operace byla zjištěna nekróza graftu kvůli dehiscenci utero-vaginální anastomózy a následnému prolapsu dělohy do pochvy s okluzí uterinních

cév (3). V průběhu následujících dvou desetiletí od tohoto pokusu se z UTx vyvinula nadějná experimentální metoda léčby neplodnosti, která by se mohla po stádiu ověřování stát v budoucnu standardní léčebnou metodou (4,5). V roce 2014 se ve Švédsku narodilo císařským řezem první dítě z dělohy transplantované od žijící dárkyně (6). Na tento porod navazovaly další, přičemž některé z příjemkyň dělohy porodily dvě děti a až poté podstoupily hysterektomii (7). První dítě z dělohy kadaverózní dárkyně se narodilo v roce 2017 v Brazílii (8).

Počátky uterinního transplantačního programu v České republice se datují do let 2014-2015, kdy proběhla studie s iniciální kohortou 50 českých pacientek s kongenitální agenezí dělohy a pochvy (syndromem Mayer-Rokitansky-Küster-Hauser) a neovaginou vytvořenou technikou podle Vecchiettiho (9). Z těchto zájemkyň o UTx se rekrutovala část pacientek, které se staly příjemkyněmi transplantované dělohy v České republice po roce 2016, přičemž 5 žen obdrželo dělohu od žijících a 5 od kadaverózních dárkyň. Celkem 7 případů UTx bylo funkčně úspěšných, všechny tyto ženy podstoupily embryotransfery v rámci asistované reprodukce a první porod zdravého dítěte po UTx proběhl v Praze ve 35. gestačním týdnu (10,11).

ETICKÉ ASPEKTY TRANSPLANTACE DĚLOHY

UTx byla již od počátku spojena s řadou kontroverzí etických, medicínských, psychologických a náboženských aspektů. Zastánci této složité léčby neplodnosti ji obhajují jako de facto jedinou kauzální terapeutickou možnost, která ženám s AUFI zajistí jak geneticky, tak zejména biologicky vlastního potomka (biologická matka je vždy ta, která dítě porodí). Ve srovnání s žijícími dárci ledvin a části jater obhájcové UTx rovněž zdůrazňují nižší riziko vzniku závažných komplikací u žijících dárkyň dělohy (12). Navíc ve srovnání se surogátním těhotenstvím, které umožňuje zajistit neplodnému páru geneticky vlastní dítě cestou náhradní těhotné, je UTx spojena s eticky akceptovatelným přijetím všech rizik spojených s otěhotněním, graviditou a porodem samotnou příjemkyní dělohy.

Kritici UTx na druhou stranu zdůrazňují zejména chirurgickou náročnost jak odběru děložního štěpu od žijící dárkyně, tak i samotné transplantace, ale upozorňují i na rizika vyplývající z užívání imunosuprese, a to za cenu "pouhého" zvýšení kvality života a nikoli za cenu jeho záchrany jako u jiných orgánových transplantací. Některé kontroverze však mohou být spojeny i s dárcovstvím dělohy od zemřelých dárkyň. Souhrn aktuálních etických postojů k této experimentální metodě léčby AUFI byl recentně publikován i v české odborné literatuře (13).

PATOLOGICKÉ ASPEKTY TRANSPLANTACE DĚLOHY

Bioptické vyšetření transplantované dělohy

Podobně jako v případě jiných orgánových transplantací je role patologa v rámci UTx nezastupitelná a spočívá zejména v hodnocení hrozících známek rejekce v transplantované děloze. Diagnostika rejekce se neobejde bez bioptického ověření, protože žádná méně invazivní metoda kontroly známek rejekce nebyla dosud objevena. Podmínkou kvalitní histopatologické diagnostiky je gynekologem technicky správně provedená biopsie z ektocervixu, ideálně pod kolposkopickou kontrolou místa optimálního odběru z děložního hrdla transplantované dělohy, což může být problémem zejména v případě potransplantační stenózy utero-vaginální anastomózy, která způsobuje horší viditelnost exocervixu nacházejícího se nad stenózou (14).

V případě UTx se kontrolní biopsie odebírá z exocervixu děložního hrdla a díky přerušení inervace dělohy při jejím odběru od dárkyně je sice pro pacientku nekomfortní, ale fakticky nebolestivá. Stále není zcela jisté, zda zánětlivé změny v exocervixu korelují s eventuálními zánětlivými (rejekčními) změnami ve zbytku dělohy, avšak nedávná švédská studie sedmi explantovaných dělohy po transplantaci potvrdila reprezentativnost exocervikálních odběrů pro celou dělohu (15). Bioptický odběr se opakuje v pravidelných intervalech po transplantaci, před otěhotněním, ale i v průběhu gravidity (4,6,8,10,16). Pokud je rejekce histopatologicky potvrzena, provádí se po podání antirejekční léčby či po úpravě udržovací imunosupresivní léčby opakovaná biopsie k vyloučení perzistence známek rejekce, což může oddálit následující embryotransfery čekáním na zhojení místa odběru tkáně z ektocervixu a zároveň i prodlužovat dobu užívání imunosupresiv.

Stanovení stupně rejekce je založeno na provizorním skórovacím systému švédských autorů, které hodnotí zánětlivý infiltrát v exocervixu (17). Tato klasifikace dělí histopatologické (subklinické) rejekční změny na mírné (grade 1), střední (grade 2) a těžké (grade 3), přičemž zároveň představuje i kategorii tzv. hraničních (borderline) změn (grade 0) známou z kontroly známek rejekce u jiných orgánových transplantací.

Provizorní skórovací systém rejekčních změn po transplantaci dělohy

V rámci současně platné klasifikace morfologie rejekčních změn se rozpoznávají 4 kategorie (17), které jsou definovány následujícími rysy a graficky znázorněny na přiloženém schématu (obr. 1).

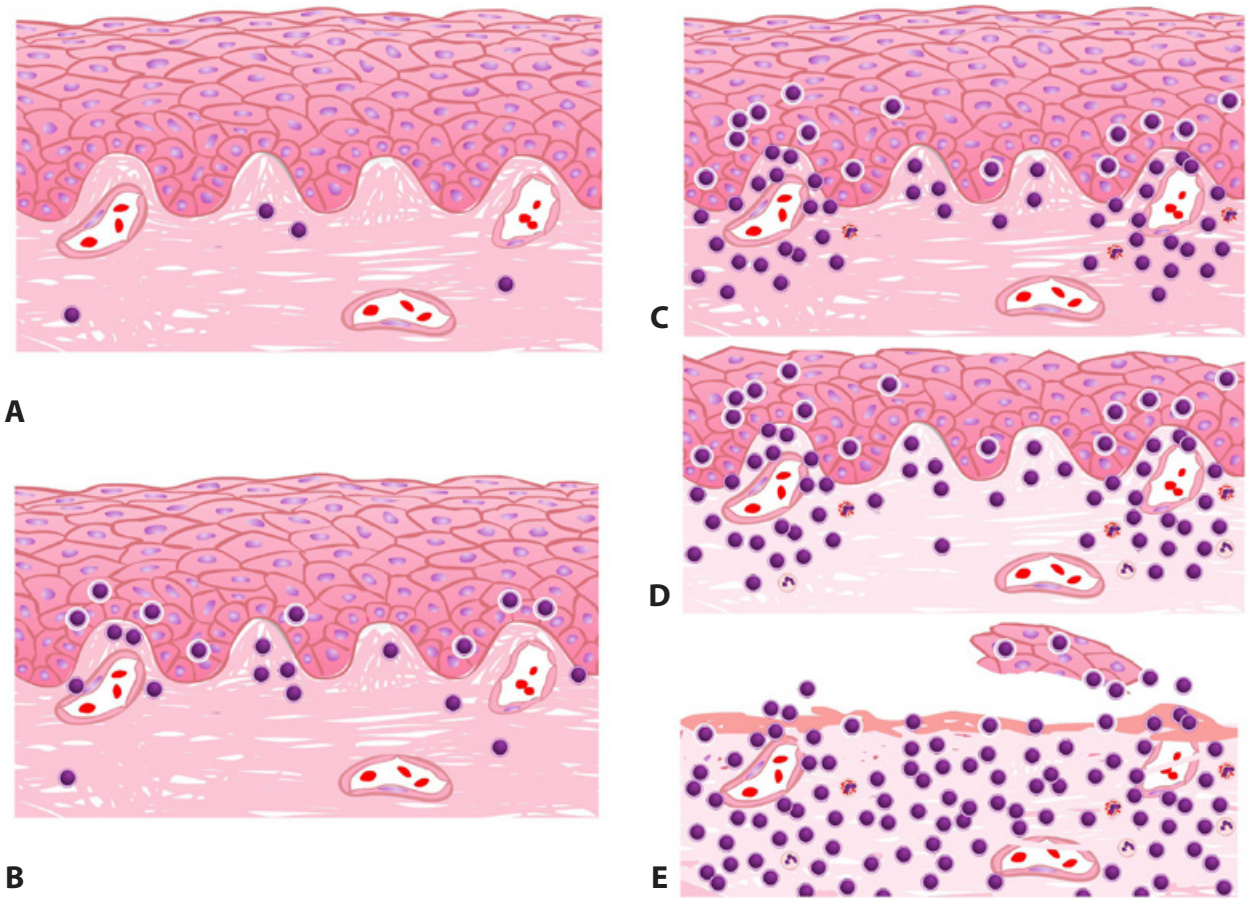
Grade 1 (mírná rejekce) je charakterizován mírným smíšeným zánětlivým infiltrátem s dominancí lymfocytů, které se nacházejí zejména na epitelo-stromální junkci exocervixu (v oblasti superficiálního stromatu a *stratum basale* dlaždicového epitelu). Zánětlivá infiltrace bývá ložisková až splývající a epitel nevykazuje regresivní změny s výjimkou ojedinělých apoptóz keratinocytů.

Grade 2 (střední rejekce) obsahuje v oblasti epitelo-stromální junkce splývající smíšený zánět s vyjádřeným intraepiteliálním influxem leukocytů. Kromě převažujících lymfocytů lze pozorovat účast četnějších neutrofilních granulocytů. Dlaždicový epitel může být fokálně ztenčený, edematózně prosáklý a obsahovat disperzní apoptotická tělíska.

Grade 3 (těžkou rejekci) definuje přítomnost nápadného difuzního smíšeného zánětlivého infiltrátu s převahou lymfocytů a účastí hojných neutrofilních a eozinofilních granulocytů. V dlaždicovém epitelu lze kromě apoptotických tělísek po zaniklých keratinocytech pozorovat eroze až ulcerace s nekrotizací výstelky i přilehlého stromatu.

Provizorní skórovací systém děložních alograftů dále uvádí kategorii tzv. hraničních (borderline) změn. Tu představují malé nesplývající aglomeráty lymfocytů, jež lze nalézt zejména v epitelo-stromální junkci. V epitelu je může doprovázet intercelulární edém; v intersticiu se nachází převážně ve stromálních papilách. Význam těchto aglomerátů ve vztahu k rejekci je zatím nejistý, jejich přítomnost byla totiž prokázána i u zdravých žen s vlastní dělohou (18).

Prozatímní klasifikace hodnotila pouze celulární rejekční změny, pro humorální rejekci dělohy stále chybí spolehlivá data. Dosud byla publikována pouze izolovaná kazuistika popisující výskyt protrahované smíšené celulární a humorální rejekce u pacientky s uterinním alograftem (19).



Obr. 1. Schéma provizorního skórovacího systému rejekčních změn po UTx.
A) Normální cervikální biopsie s izolovanými zánětlivými buňkami. **B)** Borderline změny definované shlukujícími se zánětlivými buňkami s převahou lymfocytů v oblasti epitelo-stromální junkce s intraepiteliálním influxem leukocytů. **C)** Grade 1 rejekce charakterizovaná ložiskovým smíšeným zánětlivým infiltrátem s převahou lymfocytů v oblasti epitelo-stromální junkce. **D)** Grade 2 rejekce charakterizovaná ložiskovým až splývajícím smíšeným zánětlivým infiltrátem s převahou lymfocytů v oblasti epitelo-stromální junkce, doprovázená redukcí šíře epitelu a stromálním edémem. **E)** Grade 3 rejekce s nápadným difuzním smíšeným zánětlivým infiltrátem. Epiteliální eroze/ulcerace mohou být přítomny.

ZÁVĚR

Dosud bylo ve světě provedeno přibližně 80 transplantací dělohy, díky nimž se ženám s AUFI narodilo celkem 31 dětí (20). Recentně publikovaná analýza potvrdila vysokou míru (79 %) porodů v termínu nebo krátce před stádiem zralosti a zároveň neprokázala žádné závažnější zdravotní komplikace na straně matek ani dětí, které by nebylo možné vyléčit standardními gynekologicko-porodnickými a neonatologickými léčebnými postupy (21). Ačkoli UTx čelí řadě nezodpovězených etických otázek, je vnímána jako nadějná experimentální léčba AUFI, jejíž efektivita a bezpečnost by měla být potvrzena v dohledné době po ukončení studií probíhající v mnoha zemích světa. Role patologa spočívá zejména v hodnocení rejekčních změn uterinních alograftů. Evaluace rejekcí dělohy vychází z dosud platného provizorního skórovacího systému švédských autorů (17), který však již ve svém názvu odráží nezbytnost dalšího výzkumu a možnost jeho úpravy v budoucnu. Odborná publikační aktivita zabývající se patologickými aspekty léčby neplodnosti pomocí UTx je sice minimální, ale probíhající studie by mohly v dohledné době přinést nové obohacující výsledky.

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PROHLÁŠENÍ

Autor práce prohlašuje, že v souvislosti s tématem, vznikem a publikací tohoto článku není ve střetu zájmů a vznik ani publikace článku nebyly podpořeny žádnou farmaceutickou firmou. Toto prohlášení se týká i všech spoluautorů.

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