Novel clinical and morphological predictors of malignancy in patients with ovarian endometrioid cysts

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Introduction. Endometriosis is an endless source of scientific investigations, but still the mechanisms of malignant transformation of ovarian endometriosis remain to be understood.

Patients and methods. This study was conducted on surgical specimens isolated from ovarian endometrioid cysts (OEC) and the endometriosis-associated ovarian tumors obtained after surgical operation from 117 patients. The normal level of serum CA 125 was assumed to be up to 35 IU/ml. Immunohistochemical study of MCK, CK7, CK20, CK 8/18, Calretinin, EMA, Ki67, CEA, Vimentin, Inhibin, WT1, p53, ARID1A (BAF250a), CA 125 antibodies was performed.

Results. The results revealed a direct correlation between the level of serum CA 125 and the WT1 expression in the OEC epithelium (Pearson $r=0.84$, $p<0.0001$) and between the level of serum CA 125 and the p53 expression (Pearson $r=0.81$, $p<0.0001$). A striking direct correlation was found when studying the relationship between WT1 and p53 expression in OEC epithelium (Pearson $r=0.79$, $p<0.0001$).

Conclusion. This research delineated the changes in OEC epithelium, which were similar to the serous epithelial type and associated with an extensive rise in the serum biomarker CA 125 level, which could be indicative of the early neoplastic transformation of OEC.

Keywords: CA 125, WT1, p53, ovarian endometrial cyst, ARID1A, malignant transformation

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и экспрессией p53 в эпителии ЭКЯ (r=0.81, p<0.0001). Сильная прямая корреляция обнаружена при изучении взаимосвязи между экспрессией WT1 и p53 в эпителии ЭКЯ (r=0.79, p<0.0001).

Заключение. По результатам данного исследования выявлены изменения в эпителие ЭКЯ с иммунофенотипом, подобным серозному эпителию, и эти изменения связаны со значительным повышением уровня сывороточного биомаркера CA 125, что может указывать на раннюю неопластическую трансформацию ЭКЯ.

Ключевые слова: CA 125, WT1, p53, эндометриоидная киста яичника, ARID1A, злокачественная трансформация

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Introduction

Endometriosis takes the third place in all gynecological diseases after inflammation and leiomyoma. The incidence of endometriosis in fertile women ranges up to 15% and is one of the most common causes of infertility and chronic pelvic pain syndrome [1–4].

Endometriosis is characterized by ectopic tissue morphologically similar to the endometrium, with glands and stroma. To date, many theories have been proposed, which highlight the etiology, pathogenesis, and natural history of the endometriosis and endometriosis-associated tumors with genetic alterations, changes in the microenvironment, stress factors, etc., but none of them can explain the entire variety of its forms and ability to neoplastic transformation [1, 2, 5–7].

The history of malignant transformation of endometriosis started in 1925 when John Sampson first described endometriosis-associated malignancy criteria [8]. Malignant transformation of endometriosis is a rare phenomenon that occurs in about 0.7–2.5% of cases and, when it occurs, it usually involves the ovary [1, 9, 10]. Endometriotic foci also were identified near ovarian epithelial carcinomas and within these carcinomas. So, they were named endometriosis-associated tumors and included predominantly endometrioid, clear-cell carcinomas, and a minor group of seromucinous tumors [11, 12]. The risk of endometrioid and clear-cell ovarian tumors is 2–3 times higher in women with endometriosis [10, 13]. Further investigation led to discovering so-called “atypical” endometriosis. It consumes gradual changing of normal endometrioid cyst epithelium into atypical endometriosis with loss of nuclear polarity, hyperchromic nuclei, syncytiotrophoblastic changes, large hobnail cells, and then into invasive carcinoma [10].

Endometriosis was significantly associated with borderline ovarian tumors and endometriosis-associated ovarian carcinomas [14]. Atypical endometriosis and endometriosis-associated ovarian tumors have similar molecular changes, such as PTEN, ARID1A, and HNF-1b mutations. Moreover, ARID1A mutations were observed in clear-cell tumors and atypical endometriosis but not in distant endometrioid foci [10, 15].

The mechanisms of malignant transformation of endometriosis such as genetic, epigenetic factors, microenvironment have been reported earlier [1–15]. The following neoplastic transformation pathways make a significant contribution to the development of “atypical” endometriosis and endometriosis-associated tumors. These include KRAS/BRAF mutations, TP53 mutations, mTOR pathway, ARID1A mutation, iron metabolism, redox molecules in endometriotic cyst fluid, etc. Previous investigation has revealed a number of pathophysiological changes in endometriosis-associated carcinomas, such as deregulated oxidoreductase activity, metabolism, hormone activity, inflammatory response, innate immune response, and cell-to-cell signaling [16]. DNA methylation and demethylation, histone modifications, and miRNA aberrant expressions play a significant role in endometrioid ovarian cyst (OEC) malignant transformation [17]. PI3K-AKT-mTOR, chromatin remodeling (ARID1A), Notch signaling, and Wnt/β-catenin pathway may contribute to OEC malignant transformation [1, 18–20].

Apart from the OEC malignant transformation, such cases have been reported from extraovarian endometriosis, including vaginal, ureteral, or cesarian scar. Most of the extraovarian malignancies are clear-cell or endometrioid carcinomas [21–26]. However, there are rare cases where rhabdomyosarcoma arises from OEC [27].

As previously mentioned, endometriosis-associated tumors could arise from endometrioid cysts [28]. There are reports available that cystic fluid contains several active biomolecules, such as iron, reactive oxygen species, interleukins. Iron-induced oxidative stress and DNA mutations are the basis of iron carcinogenesis, which are followed by the subsequent synthesis of the antioxidants with the decrease in cellular oxidative stress capacity, promotion of apoptosis resistance, and leading to tumor initiation and progression [29, 30]. So, with the help of metallobiology technology, these molecules can be used as non-invasive
biomarkers for detecting the endometriosis malignant transformation [31, 32].

World Health Organization approved the classification of ovarian tumors in 2014 that include high-grade and low-grade serous carcinomas (HGSC and LGSC, respectively) that differ from each other in genetic disorders, clinical course, and origin [33]. The source of HGSC is the fallopian tube epithelium implanted on the ovarian surface. It is associated with the fallopian tube’s serous intraepithelial carcinoma (STIC) and TP53 mutations [11]. LGSC is characterized by KRAS and BRAF mutations and develop sequentially from serous borderline tumors [11].

Glycosylation disorders are known as cancer markers. They lead to tumor-associated glycans and glycoproteins production. The glycosylation marker CA 125 is used for detecting and monitoring serous ovarian tumors. Since glycosylation changes are the early event in the tumor, the identification of tumor-associated markers of glycosylation is an effective strategy in early diagnosis and improving the treatment [34].

The level of serum CA 125 detected in 1% of healthy female donors can be 35 IU/ml and is often regarded as the upper limit of the normal reference in the clinical practice. It should be noted that this level is controversial, for example, in postmenopausal women or in patients after hysterectomy, the level of CA 125 tends to decrease, and the lower level may be more acceptable [35]. Approximately 85% of patients with ovarian epithelial tumors have a serum CA 125 level higher than 35 IU/ml. Serum CA 125 is less often increased in mucinous, clear-cell, and borderline tumors than in serous carcinomas. An increase in serum CA 125 level may also be associated with other malignancies (pancreas, breast, colon, lung tumors) and benign or physiological conditions, including pregnancy, endometriosis, and menstruation [35].

Regarding CA 125 as a marker of endometriosis, many authors are inclined to believe that the level of CA 125≥30 IU/ml is an indicator of endometriosis with high accuracy only in women with symptoms of pain and infertility. CA 125 should be considered a discriminatory test for suspected endometriosis, and the level of CA 125 <30 IU/ml, however, cannot be a criterion for reliable detection of endometriosis [36–39].

The present work suggested that an increased level of serum CA 125 in patients with OEC indicates a change in the morphology and physiology of endometrioid epithelium, reflecting the beginning of the neoplastic transformation of OEC.

The efficient understanding of biological and functional mechanisms depicting OEC complex pathogenesis is considered a significant aspect for developing novel diagnostic and therapeutic strategies. Furthermore, the neoplastic transformation of ovarian endometriosis remains to be understood fully despite decades of research. The present study delineated an extensive rise in the serum levels of CA 125 in OEC conditions, which indicates a change in the morphology and physiology of endometrioid epithelium conducive to the possible beginning stage of neoplasm.

**Patients and methods**

The present study was conducted on the surgical specimen samples obtained from 117 patients. 104 patients were after cystectomy with endometrioid cysts and 13 with ovarian tumors, including endometriosis-associated ones. The age group was chosen from 20 to 83 years (average 36.58±0.95 years) during the period of 2016 to 2019 admitted to the Hospital of S.S. Udina City of Moscow Hospital and the Moscow Clinical Hospital #31. All patients agreed and provided their individual informed consent to analyze their surgical specimen samples with complete Institutional ethical guidelines. Normal range of serum CA 125 was admitted under 35 IU/ml. We divided patients into five groups based on obtained data:

- group 1 includes OEC with normal levels of serum CA 125 cancer biomarker in 69 patients,
- group 2 includes an increased level of serum CA 125 (36–60 IU/ml) in 17 patients,
- group 3 includes an increased level of serum CA 125 (61–90 IU/ml) in 10 patients,
- group 4 includes an increased level of serum CA 125 (91–301 IU/ml) in 8 patients,
- and group 5 includes ovarian carcinomas, i.e., endometrioid (endometriosis-associated ones), serous low- and high-grade with solid, pseudo-endometrioid, transitional cell-like pattern (SET-type) in 13 patients.

The serum CA 125 analysis was performed at various laboratories in Moscow using immunochemiluminescent analyzers Architect 1000i (USA) and Immulite 1000 (Japan).

**Reagents**

To compare expression in OEC with ovarian tumors, we used a diagnostic panel of mouse monoclonal antibodies against MCK, CK7, CK20, CK 8/18, Calretinin, EMA, Ki67, CEA, Vimentin, Inhibin, WT1, CA 125, ARID1A (BAF250a) (Santa Cruz, USA).

**Immunohistochemistry**

OEC and endometriosis-associated tumors surgical specimens were collected into buffered neutral 10% formalin and subjected to fixation. Paraaffin-embedded tissue blocks were prepared through routine histological processing. Immunohistochemical analysis was executed with the aid of the avidin-biotin complex technique. Histological sections were made using Sakura rotary microtomes, stained with hematoxylin and eosin. Immunohistochemistry study of MCK, CK7, CK20, CK 8/18, Calretinin, EMA, Ki67, CEA, Vimentin, Inhibin, WT1, CA 125, ARID1A (BAF250a) antibodies was performed using “Leica BondMax” immunostainers (Germany).

Interpretation of the immunohistochemical results was carried out considering the localization of positive cells by counting both the number of colored epithelial cells
and chronic endometritis. Three (3) patients had a history of breast fibroadenoma (2.9%). Twenty-five (25) (24%) patients had no combined gynecological pathology, and 20 (19%) patients had more than two diseases (Fig. 1). Gynecological diseases combined with endometriosis-associated tumors (group 5) included leiomyoma in 1 patient (7.7%).

**Combined gynecological diseases in OEC patients**

Expression of MCK, CK7, CK8/18, and EMA markers in OEC and endometriosis-associated ovarian tumors did not differ in all groups and were expressed in all cases. Expression of CK20, CEA, Calretinin, Inhibin, and Vimentin in OEC epithelium and endometriosis-associated ovarian tumors was negative. The expression of proliferation marker Ki67 in OEC did not exceed 5% in all cases. In contrast, its expression in ovarian tumors was 10–15% in endometrioid and low-grade serous carcinomas and 50–71% in high-grade serous carcinoma (SET-type). Expression of the immunohistochemical marker CA 125 was higher in serous ovarian tumors and in the OEC group with elevated serum CA 125 levels above 60 IU/ml. ARIDIA (BAF250a) expression was 70-96% (average 83.73±0.67%) in all groups of OEC, 10–43% (average 21.6±4.5%) in endometrioid ovarian carcinomas and 60–67% (average 64±1.4%) in serous carcinomas low- and high-grade (Table 1).

Significant changes in nuclear expression were detected when evaluating the immunohistochemical reaction for WT1 and p53 expression. The WT1 gene located on chromosome 11p13 provides protein synthesis required for the development of normal kidneys and gonads. WT1 protein is a transcription factor significantly involved in the growth, maturation, cell differentiation, and apoptosis in these tissues. The characteristic feature for serous differentiation of OEC epithelial cells is an intense nuclear expression of WT1 both in individual cells and in a group of epithelial cells.

**Fig. 1. Combined gynecological diseases in OEC patients**

Рис. 1. Комбинированные гинекологические заболевания у пациенток с ЭКЯ
The nuclear expression of WT1 in the OEC epithelium was predominantly negative in the patients of 1–2 group with serum CA 125 levels up to 60 IU/ml (0–35; 36–60 IU/ml). However, a few positive nuclear expression cases of WT1 were observed in individual epithelial cells and were up to 6.5% (average 0.4±0.1%). Positive nuclear expression was observed in stromal cells in all cases in all groups and was 41–78.3% (average 67.9±5.6%). Patients with elevated levels of serum CA 125 61–90 IU/ml showed a positive nuclear expression up to 58% (average 20.7±6.4%) of OEC epithelial cells. Patients with elevated levels of serum CA 125 91–301 IU/ml demonstrated the positive nuclear expression in 62–86% epithelial cells (average 77.0±3.01%).

The expression of protein p53 was predominantly wild-type, while the percentage of nuclear expression in

### Table 1 | Таблица 1

| Group | Экспрессия IHC маркеров во всех группах |
|-------|----------------------------------------|
| Group | MCK | CK7 | CK8/18 | EMA | Ki-67 | WT1 | p53 | CA125 | ARID1A BAF250 |
|-------|-----|-----|--------|-----|-------|------|-----|-------|----------------|
| 1 (0–35 UE/ml) | 3+ | 3+ | 3+ | 3+ | 1–2% | 0–2% | Wild-type | Дикий тип, 0–15% | 1–2+ | 3+ |
| 2 (36–60 UE/ml) | 3+ | 3+ | 3+ | 3+ | 1–2% | 0–6.5% | Wild-type | Дикий тип, 1–54% | 1–2+ | 3+ |
| 3 (61–90 UE/ml) | 3+ | 3+ | 3+ | 3+ | 2–3% | 0–58% | Wild-type | Дикий тип, 3–60% | 2–3+ | 3+ |
| 4 (91–301 UE/ml) | 3+ | 3+ | 3+ | 3+ | 2–4% | 62–86% | Mutant-type | Мутантный тип, 14–93% | 2–3+ | 3+ |

**SET-type** – серозная карцинома high-grade с солидными, эндометриоидными и переходноклеточными участками строения.
Table: Serum CA 125 level, IU/ml | Уровень сывороточного CA 125, МЕ/мл

- Under 60 IU/ml | Менее 60 МЕ/мл
- More than 60 IU/ml in negative WT1 expression areas | Свыше 60 МЕ/мл в областях с негативной экспрессией WT1
- More than 60 IU/ml in positive WT1 expression areas | Свыше 60 МЕ/мл в областях с положительной экспрессией WT1

Fig. 3. WT1, p53, and ARID1A (BAF250a) expression in OEC epithelial and stromal cells
Рис. 3. Экспрессия WT1, p53 и ARID1A (BAF250a) в эпителиальных и стромальных клетках ЭКЯ
OEC epithelial cells gradually but unevenly increased with increasing level of serum CA 125 and ranged from 0 to 75% (average 13.77±2.21%) in 99 cases. The expression level of p53 was over 80% in 4 cases with serum CA 125 levels 94.3-301 IU/ml, which is an indicator of pathological expression of p53 (Fig. 3, 4). P53 expression was predominantly wild-type, and the percentage of expression was 15–82% (average 33.56±4.6%) in endometrioid and serous low-grade ovarian tumors, and the mutant-type expression was detected in high-grade serous carcinoma (SET-type).

**WT1 and p53 expression in OEC epithelial cells**

Statistical analysis of this data was completed. We have identified a strong direct correlation between the serum CA 125 level and the WT1 expression level in the OEC epithelium (Pearson correlation coefficient \( r = 0.84, p<0.0001 \)) and between the serum CA 125 level and p53 expression (Pearson, \( r = 0.81, p<0.0001 \)). There was a moderate inverse relationship between the serum CA 125 level and the WT1 expression level in the OEC stroma (Pearson, \( r = -0.68, p = 0.002 \)) and between WT1 expression in the epithelium and stroma of OEC (Pearson, \( r = -0.69, p = 0.5 \)).

When studying the relationship between the expression of WT1 and p53 in the OEC epithelium, a strong direct correlation was revealed (Pearson, \( r = 0.79, p<0.0001 \)), which may be associated with early neoplastic transformation.

BMI and OEC diameter were also evaluated in this study [40, 41]. Studies of the Pearson correlation coefficient for samples over 60 were conducted, and nonparametric statistics such as the Mann–Whitney coefficient (U) and Spearman correlation coefficient (P) were evaluated. When evaluating the Mann-Whitney coefficient, no differences were found in the samples in groups I–II and V. Significant differences in the samples were found in groups III and IV. At the same time, there was no significant correlation between the size of the OEC, BMI, and the expression of CA125, WT1, and p53 in groups I–II (0–60 IU/ml), the Spearman coefficient ranges −0.02–0.4, which indicates a weak direct/inverse correlation. In group III (61–90 IU/ml), a moderate direct relationship was found between the OEC size and the expression of the immunohistochemical markers WT1 and p53 and a moderate inverse relationship between BMI and the level of p53 expression. There was a moderate direct relationship between the OEC size and the level of serum CA 125, as well as the level of p53 expression, and a moderate inverse relationship between BMI and WT1 expression in group IV (91–301 IU/ml). A moderate inverse relationship between BMI and WT1 expression was also found in the group of ovarian tumors. Summary data of parametric and nonparametric correlation in OEC groups are in Table 2a, 2b.

**Table 2a**

| Summary data of correlation in all OEC groups (Pearson, \( r \)) | Сводные корреляционные данные во всех группах ЭКЯ (критерий Пирсона, \( r \)) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Serum CA 125, IU/ml | Уровень CA 125 в сыворотке крови, МЕ/мл | Body mass index, kg/m² | Индекс массы тела, кг/м² | OEC diameter, mm | Диаметр ЭКЯ, мм | P53 expression in epithelium, % | Экспрессия P53 в эпителии, % | WT1 expression in epithelium, % | Экспрессия WT1 в эпителии, % |
| Serum CA 125, IU/ml | Уровень CA 125 в сыворотке крови, IU/ml | – | –0,14 | 0,9 | 0,81 | 0,84 |
| Body mass index, kg/m² | Индекс массы тела, кг/м² | –0,14 | – | –0,06 | –0,14 | –0,11 |
| OEC diameter, mm | Диаметр ЭКЯ, мм | 0,09 | –0,06 | – | 0,14 | 0,11 |
| P53 expression in epithelium, % | Экспрессия P53 в эпителии, % | 0,81 | –0,14 | 0,14 | – | 0,79 |
| WT1 expression in epithelium, % | Экспрессия WT1 в эпителии, % | 0,84 | –0,11 | 0,11 | 0,79 | – |
Discussion

CA125 is a significant marker, and it is mostly used to identify disease conditions, including OEC. Predominantly, the CA125 levels are reported to be enhanced in 80–85% in females with advanced stages of ovarian cancer and endometriosis. Hence, it is referred to as a suitable cancer marker to monitor ovarian tumor progression, differentiation, and regression to foster early diagnosis [38]. A plethora of research studies established a positive link between endometriosis and certain groups of ovarian tumors, such as endometrioid, clear-cell, and serous low-grade, based on data from more than 21,000 patients [42]. Yiying Wang et al. reported that the immunohistochemical expression of markers OVGP1, WT1, and FMO in LGSCs and concluded these carcinomas develop from tubal-type epithelium of ovarian cystic inclusions [43, 44]. Abnormal expression of the WT1 protein is well known as a serous epithelium of ovarian cystic inclusions [43, 44].

According to our data, there was focal serous differentiation of OEC epithelium with expression of serous markers such as WT1 and p53 with an increase in the CA 125 level more than 60 IU/ml. We found that the IHC-profile for normal endometrioid epithelium was WT1/p53wt/ARID1A+. Moreover, when we compare the IHC-profile of OEC group 3 (61–90 IU/ml) with group 5 (endometriosis-associated tumors), we stated their equality, i.e., WT1+/p53wt/ARID1A+. Moreover, when we compare the IHC-profile of group 4 (91–301 IU/ml) to group 5, these were similar: WT1+/p53wt/ARID1A+. These findings suggest the neoplastic transformation to serous ovarian borderline tumors and LGSC. We found OECs share similar immunohistochemical profiles with serous ovarian carcinomas, but not with the endometrioid ones because the expression of WT1 and p53 were not observed, and there was no lack of ARID1A expression. The expressions of WT1 and p53 levels were focal across the endometrioid carcinomas. Besides, ARID1A (BAF250a) expression in OEC was close to that in serous low-grade carcinomas and differed from endometrioid ones. Moreover, there is a clinical case that demonstrated OEC malignant transformation to well-differentiated endometrioid carcinoma with normal serum CA 125 [50], which supports our supposition that OEC may transform to serous ovarian tumor, and this process is accompanied by an increase in serum CA 125 level.

Bulun et al. established that endometriotic stromal cells are mutation-free. We likewise found that the stromal cells did not display any changes in immunohistochemical marker expression. These authors have depicted that the stroma cells contain widespread epigenetic defects, which could alter gene expression and induce a progesterone-resistance and inflammation. Subsequent estrogenic action in the stroma results in paracrine signaling to neighboring epithelial cells, which may enhance proliferation, causing mutation accumulation and malignant transformation in OEC epithelial cells that may lead to epithelial ovarian cancer [51].
Fig. 5. Possible mechanisms of neoplastic transformations of OEC. OIC – ovarian inclusion cyst; OEC – ovarian endometrioid cyst; OSC – ovarian serous cystadenoma; RM – retrograde menstruation; ROS – reactive oxygen species; SE – serous epithelium; OVG1 – oviduct-specific glycoprotein 1; WT1 – Wilm’s tumor protein; FMO3 – flavin-containing monoxygenase 3; ARID1A – AT-rich interactive domain-containing protein 1A; PIK3CA – phosphatidylinositol-3-kinase, catalytic subunit; KRAS – RAS/MAPK pathway protein; BRAF – v-RAF murine sarcoma viral oncogene homolog b; PTEN – homolog of phosphatase and tensin; IL-1 – interleukin 1; IL-6 – interleukin 6; PGE2 – prostaglandin E2; MIF – macrophage migration inhibitory factor; p53 – p53 protein; CA 125 – cancer antigen 125

In this regard, a scheme for the possible development of ovarian carcinomas from OEC was proposed (Fig. 5). Our results reported that OEC could be precursors to both endometrioid carcinomas via the ARID1A, PI3K/AKT, MAPK/ERK pathways, and serous carcinomas via the TP53, RAS/MAPK pathways.

Conclusion
Evaluation of immunohistochemical expression of WT1 and p53 markers in the OEC with increased serum CA 125 level is beneficial to oncologists to choose a suitable therapeutic modality with adequate follow-up to the patients. Our present study results replicate previously published reports. Furthermore, the results indicate an early change in the epithelium that may give rise to neoplastic transformation is feasible. However, the mechanism of neoplastic transformation remains only partially understood and requires extensive research.

Human and animal rights
All the study procedures were approved by the US NIH Ethical Standards. Animals were not used for this study. Consent was obtained from all the humans in this study. The procedures were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the NIH ethical guidelines.

Compliance with ethics guidelines
This article is based on research that was ethically approved by the Local Ethical Committee of the City Clinical Hospital No. 31, Moscow, and Institute of Human Morphology, Moscow, Russia (Approval Protocol No. 3, 12.06.2019).

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Each author has participated sufficiently in work to take public responsibility for the content as per ICMJE guidelines; authorship credit should be based on substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work.

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Каждый автор в достаточной степени участвовал в работе, чтобы вынести ее на всеобщее обозрение, взять ответственность за ее содержание в соответствии с руководящими принципами ICMJE; признание авторства основывается на существенном вкладе в разработку концепции и дизайна; сборе, анализе и интерпретации данных; написании статьи или ее критическом рецензировании и внесении правок, имеющих решающее значение для интеллектуального содержания в соответствии с руководящими принципами ICMJE; вынести ее на всеобщее обозрение, взять ответственность за ее содержание в соответствии с руководящими принципами ICMJE; признание авторства основывается на существенном вкладе в разработку концепции и дизайна; сборе, анализе и интерпретации данных; написании статьи или ее критическом рецензировании и внесении правок, имеющих решающее значение для интеллектуального содержания; окончательном утверждении версии для публикации и согласии нести ответственность по всем аспектам работы.

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