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RESEARCH ARTICLE

Clinical laboratory features of Meigs' syndrome: a retrospective study from 2009 to 2018

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Abstract **Meigs' syndrome (MS), a rare complication of benign ovarian tumors, is easily misdiagnosed as ovarian cancer (OC). We retrospectively reviewed the clinical laboratory data of patients diagnosed with MS from 2009 to 2018. Serum carbohydrate antigen 125 and HE4 levels were higher in the MS group than in the ovarian thecoma-fibroma (OTF) and healthy control groups (all $P < 0.05$). However, the serum HE4 levels were lower in the MS group than in the OC group ($P < 0.001$). A routine blood test showed that the absolute counts and percentages of lymphocytes were significantly lower in the MS group than in the OTF and control groups (all $P < 0.05$). However, these variables were higher in the MS group than in the OC group (both $P < 0.05$). The neutrophil-to-lymphocyte ratio (NLR) was also significantly lower, whereas the lymphocyte-to-monocyte ratio was higher in the MS group than in the OC group (both $P < 0.05$). The NLR, platelet-to-lymphocyte ratio, and systemic immune index were significantly higher in the MS group than in the OTF and control groups (all $P < 0.05$). The hypoxia-inducible factor-1 mRNA levels were also significantly higher, whereas the glucose transporter 1, lactate dehydrogenase, and enolase 1 mRNA levels were lower in peripheral $CD4^+$ T cells obtained preoperatively in a patient with MS than those in patients with OTF, patients with OC, and controls (all $P < 0.05$). The expression of these four glucose metabolism genes was preferentially restored to normal levels after the tumor resection of MS ($P < 0.001$). These clinical laboratory features can be useful in improving the preoperative diagnostic accuracy of MS.**

Keywords Meigs' syndrome; ovarian thecoma-fibroma; NLR (neutrophil to lymphocyte ratio); $CD4^+$ T cells; glucose metabolism

Introduction

Ovarian thecoma-fibroma (OTF) is a type of benign ovarian tumor with histological features of fibroma and thecoma. OTF is mainly diagnosed in postmenopausal women and accounts for 1%–4% of all types of ovarian tumors [1–3]. Meigs' syndrome (MS) is a rare complication in only 1%–10% of OTF and accompanied by ascites and/or pleural effusion and rapidly disappears after tumor resection [4,5]. Given the lack of characteristic complaints and signs of MS, the nonspecific tumor marker serum carbohydrate antigen 125 (CA125) and the diagnostic value of imaging are not ideal. Therefore, differentiating MS from other ovarian tumors is difficult, especially ovarian cancer (OC) before surgery. However, owing to the low incidence and few cases of MS, information on the clinical laboratory features of this syndrome is lacking. Therefore, in this retrospective study, we analyzed the clinical laboratory indicators and expression levels of glucose metabolism genes in the peripheral $CD4^+$ T cells of patients with MS, aiming to improve the preoperative diagnostic accuracy of MS.

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Materials and methods

Patients and clinical data

This research was approved by the Ethical Committee of the First Affiliated Hospital of Nanjing Medical University

(Nanjing, China). We recruited 90 patients with OTF from 2009 to 2018 and 40 patients with OC, which were confirmed by pathology, and 42 age-matched healthy controls. None of the patients received radiotherapy or chemotherapy before collecting peripheral blood. Among these patients, nine patients with OTF were defined as the MS group according to the following diagnostic criteria: the existence of an OTF solid tumor with ascites and/or pleural effusion and ascites resolved after the tumor was quickly resected. The remaining 81 patients comprised the OTF group. Previous clinical information and pathological, imaging, and clinical laboratory results was collected for analysis.

Blood sample collection and peripheral $CD4^+$ T-cell isolation

Fresh peripheral blood samples were collected from a patient with MS and a patient with OTF before and after surgery and from nine patients with OC before surgery and 10 age-matched controls. Peripheral blood mononuclear cells were isolated by Ficoll–Paque PLUS density gradient centrifugation (GE Healthcare Bio-Sciences, Sweden). $CD4^+$ T cells were then separated with a CD4-positive isolation kit (Miltenyi Biotec, Germany). The isolated $CD4^+$ T-cell purity was higher than 95%, as determined by flow cytometry.

RNA extraction and real-time PCR

Total RNA, which was extracted from $CD4^+$ T cells using an RNeasy Micro Kit (Qiagen, USA), was reverse-transcribed into cDNA with a PrimeScript RT Reagent Kit (TaKaRa Bio, Japan). The expression levels of the four glucose metabolism genes, namely, hypoxia-inducible factor-1 (HIF1α), glucose transporter 1 (GLUT1), lactate dehydrogenase (LDHα), and enolase 1 (ENO1) were determined with an ABI 7500 real-time PCR system (Applied Biosystems, USA) using SYBR Green (TaKaRa Bio, Japan). Each experiment was performed in triplicate. The relative expression levels of the target genes were normalized by β-actin and calculated using $2^{-\Delta Ct}$. The sequences of the primers are listed in Table 1.

Statistical analysis

SPSS 20.0 software (IBM Corp, Armonk, NY, USA) was used in analyzing data. The Student's *t*-test was used to compare data from the same patient before and after the operation. The differences between the groups were determined by the Mann–Whitney U test. The chi-squared test was used in comparing rates, and Pearson's correlation was used in analyzing the relationships. All data were presented as median ± interquartile range (M ± IQR), except the expression levels of the four genes, which were shown as mean ± standard error of the mean (M ± SEM). A P value of < 0.05 was considered statistically significant.

Results

Clinical characteristics of patients with MS

The incidence of OTF was 2.24% (95/4242) in our hospital from 2009 to 2018. The difference in age among the MS, OTF, and OC groups was nonsignificant. The clinical manifestations of MS were nonspecific and included abdominal distention, poor appetite, and weight loss as common presenting symptoms. An imaging examination of one patient with MS is shown in Fig. 1. Computed tomography (CT) showed a lesion with a diameter of 14.8 cm × 11.3 cm and presented heterogeneous density, slight enhancement, and massive ascites of > 1000 mL. Magnetic resonance imaging (MRI) showed a lesion with a maximum size of 13 cm × 11 cm, with iso-intensity on T1-weighted images and a heterogeneous signal on T2-weighted images. The amount of ascites was large and exceeded 1000 mL. However, the clinical manifestations and imaging results can lead to the misdiagnosis of an ovarian malignant tumor. Therefore, laparotomy was performed on all the patients in the MS group for clear clinical diagnosis, whereas more than half of the patients in the OTF group had laparoscopic surgery (100% vs. 44.44%, $P < 0.01$, Table 2). Pathological diagnosis showed that all tumors were even larger than 10 cm in the MS group compared with the OTF group (100% vs. 8.64%, $P < 0.001$, Table 2). Pathological diagnosis showed that 52 (57.78%) of the patients had ovarian

Table 1 Sequence of primers for four glucose metabolism genes

Genes	Forward primer (5′→3′)	Reverse primer (5′→3′)
HIF1α	CCATTAGAAAGCAGTTCCGC	TGGGTAGGAGATGGAGATGC
GLUT1	TTGGCTCCGGTATCGTCAAC	GCCAGGACCCACTTCAAAGA
LDHα	CCAGCGTAACGTGAACATCTT	CCCATTAGGTAACGGAATCG
ENO1	TCATCAATGGCGGTTCTCA	TTCCCAATAGCAGTCTTCAGC
β-actin	GAGCTACGAGCTGCCTGACG	GTAGTTTCGTGGATGCCACAG

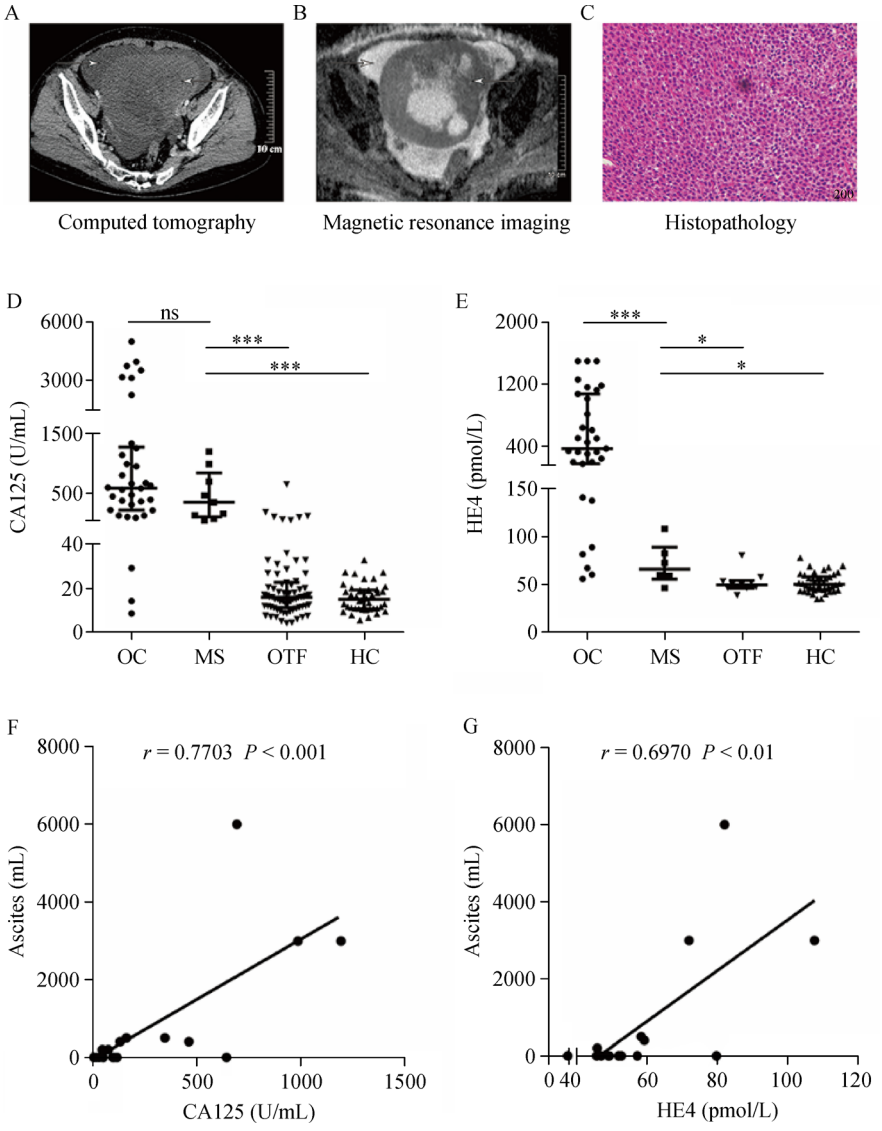

Fig. 1 Clinical imaging and pathological findings, and serum CA125 and HE4 levels in patients with MS. (A, B) CT scan and MRI examination show a lesion with a diameter of > 10.0 cm × 10.0 cm, accompanied by massive ascites (> 1000 mL). The left arrow indicates ascites, and the right arrow represents the tumor. (C) The pathological type is fibroma, as shown by hematoxylin and eosin staining (200×). (D) Serum CA125 levels in the MS group ($n = 9$) compared with those in the OC ($n = 34$), OTF ($n = 70$), and control groups ($n = 42$). (E) Serum HE4 levels in the MS group ($n = 6$) compared with those in the OC ($n = 31$), OTF ($n = 10$), and control groups ($n = 42$). (F, G) Correlations of serum CA125 and HE4 levels with the amount of ascites. Data are shown as median ± IQR. *$P < 0.05$ and ***$P < 0.001$.

fibroma, 33 (36.67%) had ovarian fibrothecoma, and 5 (5.56%) had ovarian thecoma.

Serum levels of tumor markers CA125 and human epididymis protein 4 in patients with MS

CA125 and human epididymis protein 4 (HE4) levels were significantly higher in the MS group than in the OTF and control groups (CA125: 347.6 ± 739 U/mL vs. 15.93 ± 11.48 U/mL vs. 15.0 ± 8.65 U/mL, both $P < 0.001$ and HE4: 65.66 ± 33.25 pmol/L vs. 49.23 ± 8.14 pmol/L vs. 49.73 ± 14.13 pmol/L, both $P < 0.05$; Fig. 1). The difference in CA125 levels between the MS and OC groups was nonsignificant, but the HE4 levels were significantly lower in the MS group than in the OC group (65.66 ± 33.25 pmol/L vs. 365.8 ± 901.9 pmol/L, $P < 0.001$). The serum CA125 and HE4 levels were positively correlated with the amount of ascites ($P < 0.001$ and $P < 0.01$, respectively). Therefore, the combination of CA125 and HE4 could not distinguish MS from other ovarian tumors easily and may lead to misdiagnosis.

Table 2 Clinical characteristics of patients with MS

Parameters	MS group (n = 9)	OTF group (n = 81)	P value
Age (year)	53.78±9.30	52.33±12.90	0.745
Surgical procedures			0.003
Laparotomy	9 (100%)	36 (44.44%)	
Laparoscopy	0	45 (55.56%)	
Tumor sizes			<0.001
<10 cm	0	74 (91.36)	
⩾10 cm	9 (100%)	7 (8.64%)	
Pathologic types			0.697
Fibroma	5 (55.56%)	40 (49.38%)	
Fibrothecoma	4 (44.44%)	37 (45.68%)	
Thecoma	0	4 (4.94%)	

Features of peripheral blood cells in patients with MS

We examined the counts of peripheral blood cells by evaluating their features in MS. The absolute count of lymphocytes ($(1.62 \pm 0.33) \times 10^9$/L vs. $(1.96 \pm 0.80) \times 10^9$/L vs. $(1.85 \pm 0.54) \times 10^9$/L, both $P < 0.05$) and the percentage of lymphocytes ($27.5\% \pm 9.95\%$ vs. $34.5\% \pm 11.9\%$ vs. $35.0\% \pm 10.1\%$, both $P < 0.05$) were significantly lower in the MS group than in the OTF and control groups. However, the two lymphocyte measures were significantly higher in the MS group than in the OC group ($(1.62 \pm 0.33) \times 10^9$/L vs. $(1.31 \pm 0.63) \times 10^9$/L and $27.5\% \pm 9.95\%$ vs. $20.4\% \pm 11.9\%$, $P < 0.01$ and $P < 0.05$, respectively; Fig. 2). Other peripheral blood indicators, including the absolute counts of the white blood cells, monocytes, neutrophils, and platelets and the percentages of monocytes and neutrophils, were not significantly different between the MS and OC groups. However, the percentages of neutrophils ($65.7\% \pm 12.6\%$ vs. $57.0\% \pm 13.7\%$ vs. $55.3\% \pm 11.5\%$; $P < 0.05$ and $P < 0.01$, respectively) and absolute count of platelets ($(303 \pm 79.0) \times 10^9$/L vs. $(205 \pm 55.0) \times 10^9$/L vs. $(210.5 \pm 63.3) \times 10^9$/L, $P < 0.001$ and $P < 0.01$, respectively) were significantly higher in the MS group than in the OTF and control groups. These data indicated

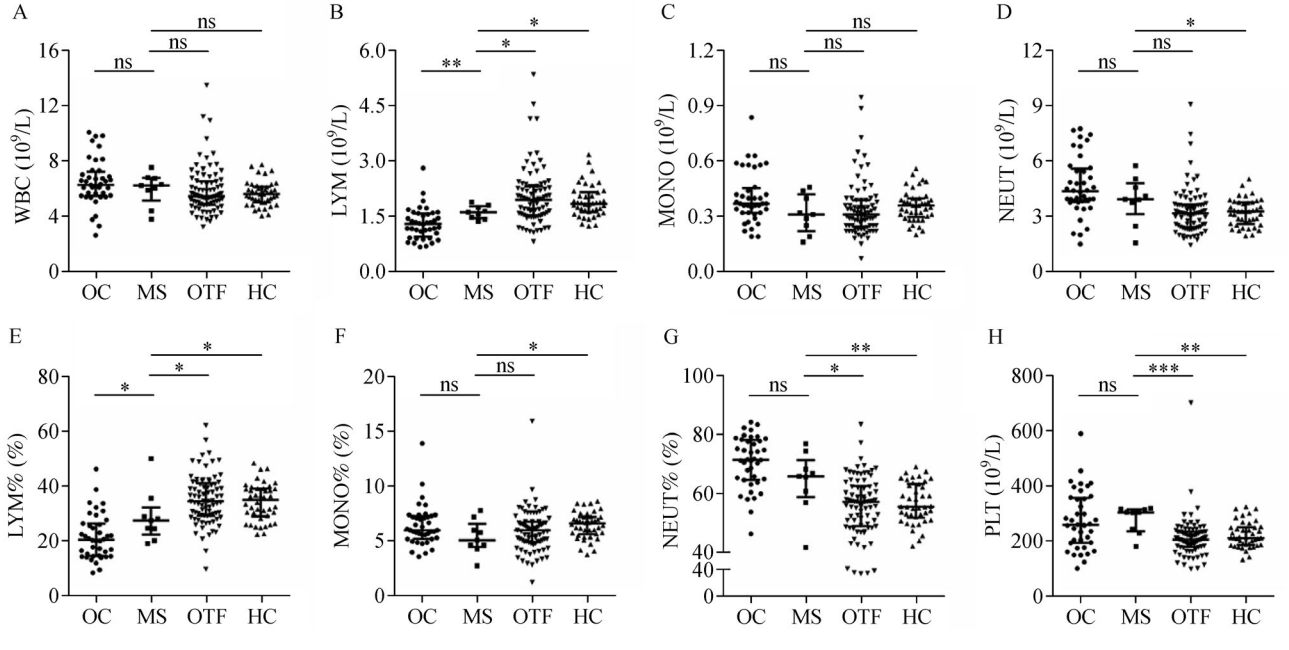

Fig. 2 Features of peripheral blood cells in the MS group. (A–D, H) Absolute counts of white blood cells (WBC), lymphocytes, monocytes, neutrophils, and platelets in the MS group (n = 9) compared with the OC group (n = 40), OTF group (n = 81), and control group (n = 42). (E, F, G) Percentages of lymphocytes (LYM%), monocytes (MONO%), and neutrophils (NEUT%) in the MS group (n = 9) compared with the OC group (n = 40), OTF group (n = 81), and control group (n = 42). Data are shown as median ± IQR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

that lymphocytes were in a dominant position and may result in the peripheral immune dysfunction of the MS.

Levels of systemic inflammatory indicators in patients with MS

Given the altered distribution of the peripheral blood cells in MS, we examined the features of systemic inflammatory indicators, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), and systemic immune index (SII = NLR × platelets). These indicators can reflect systemic inflammatory and immune responses by combining the four types of the circulating immune-inflammatory cells (lymphocytes, monocytes, neutrophils, and platelets). The NLR (2.43 ± 1.37 vs. 3.44 ± 2.77, $P < 0.05$) was significantly lower, and the LMR (4.47 ± 4.07 vs. 3.32 ± 1.93; $P < 0.01$) was higher in the MS group than in the OC group, although the PLR and SII were not significantly different between these groups (Fig. 3). The NLR (2.43 ± 1.37 vs. 1.65 ± 0.98 vs. 1.58 ± 0.90, both $P < 0.05$), PLR (183.0 ± 57.0 vs. 101.9 ± 47.6 vs. 109.7 ± 53.4, both $P < 0.001$), and SII (759.3 ± 395.4 vs. 338.3 ± 172.8 vs. 343.4 ± 262.7; $P < 0.001$ and $P < 0.01$, respectively) were significantly higher in the MS group than in the OTF and control groups. These findings suggested that an imbalance in the peripheral immune system in patients with MS and combination of the four systemic inflammatory indicators were responsible for the differential diagnosis.

Expression levels of glucose metabolism genes in peripheral $CD4^+$ T cells of patients with MS

Given the changes in lymphocytes in peripheral blood and key role glucose metabolism in $CD4^+$ T cells, we examined mRNA expression of four glucose metabolism genes in peripheral $CD4^+$ T cells in one patient with MS and in one patient with OTF before and after operation and in nine patients with OC before surgery and 10 controls. The preoperative HIF 1α mRNA levels in the patient with MS were significantly higher than those in the patient with OTF, patients with OC, and controls ($1.088 \pm 1.112 \times 10^{-2}$ vs. $0.1562 \pm 3.123 \times 10^{-3}$ vs. $0.4270 \pm 5.607 \times 10^{-2}$ vs. $7.229 \times 10^{-2} \pm 9.186 \times 10^{-3}$; $P < 0.001$, $P < 0.01$, and

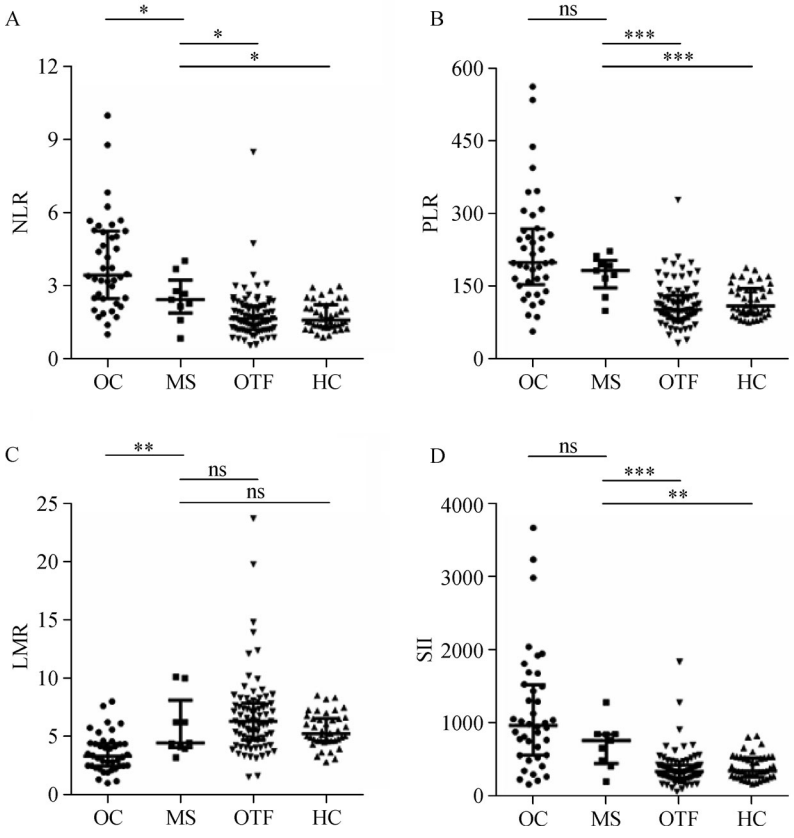

Fig. 3 Levels of systemic inflammatory indicators in the MS group. (A–D) The neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR), and systemic immune index (SII = NLR × platelets) in the MS group ($n = 9$) compared with the OC group ($n = 40$), OTF group ($n = 81$), and control group ($n = 42$). Data are shown as median ± IQR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

$P < 0.01$, respectively; Fig. 4). GLUT1 mRNA levels were significantly lower in the preoperative patient with MS than in the patient with OTF, patients with OC, and controls ($3.374 \times 10^{-4} \pm 2.700 \times 10^{-5}$ vs. $3.776 \times 10^{-3} \pm 7.552 \times 10^{-5}$ vs. $1.687 \times 10^{-2} \pm 2.635 \times 10^{-3}$ vs. $6.888 \times 10^{-3} \pm 1.012 \times 10^{-3}$; $P < 0.001$, $P < 0.01$, and $P < 0.01$, respectively). LDHα mRNA levels were significantly lower in the preoperative patient with MS than in the patient with OTF, patients with OC, and controls ($2.389 \times 10^{-2} \pm 3.472 \times 10^{-3}$ vs. $3.659 \times 10^{-2} \pm 7.318 \times 10^{-4}$ vs. $0.2219 \pm 4.203 \times 10^{-2}$ vs. $6.560 \times 10^{-2} \pm 1.019 \times 10^{-2}$; $P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively). ENO1 mRNA levels were significantly lower in the patient with MS than in patients with OC and controls ($5.239 \times 10^{-3} \pm 4.366 \times 10^{-4}$ vs. $0.1114 \pm 2.158 \times 10^{-2}$ vs. $6.323 \times 10^{-2} \pm 6.600 \times 10^{-3}$, both $P < 0.01$). We further compared expression of these four genes in one patient with MS before and after surgery. Postoperatively, the patient with MS had a significantly lower HIF1α mRNA level compared with that obtained preoperatively ($0.3272 \pm 7.953 \times 10^{-3}$ vs. $1.088 \pm 1.112 \times 10^{-2}$, $P < 0.001$). However, GLUT1 ($2.080 \times 10^{-3} \pm 5.132 \times 10^{-5}$ vs. $3.37 \times 10^{-4} \pm 2.700 \times 10^{-5}$, $P < 0.001$), LDHα ($0.3453 \pm 2.322 \times 10^{-3}$ vs. $2.389 \times 10^{-2} \pm 3.472 \times 10^{-3}$, $P < 0.001$), and ENO1 ($4.842 \times 10^{-2} \pm 4.203 \times 10^{-4}$ vs. $5.239 \times 10^{-3} \pm 4.366 \times 10^{-4}$, $P < 0.001$) mRNA levels were relatively higher postoperatively. Therefore, the expression levels of the four glucose metabolism genes, namely, HIF1α, GLUT1, LDHα, and ENO1, were all restored to normal levels after surgical removal of the tumor. These results suggested that the expression levels of the four glucose metabolism genes in peripheral $CD4^+$ T cells that relate to tumor pressure may be the potential indicators of MS.

Discussion

MS was originally reported and named by professor Meigs in 1937 [6]. This disease has attracted wide attention because it is a benign ovarian tumor that is complicated by many ascites and pleural effusion [4,7]. Clinical

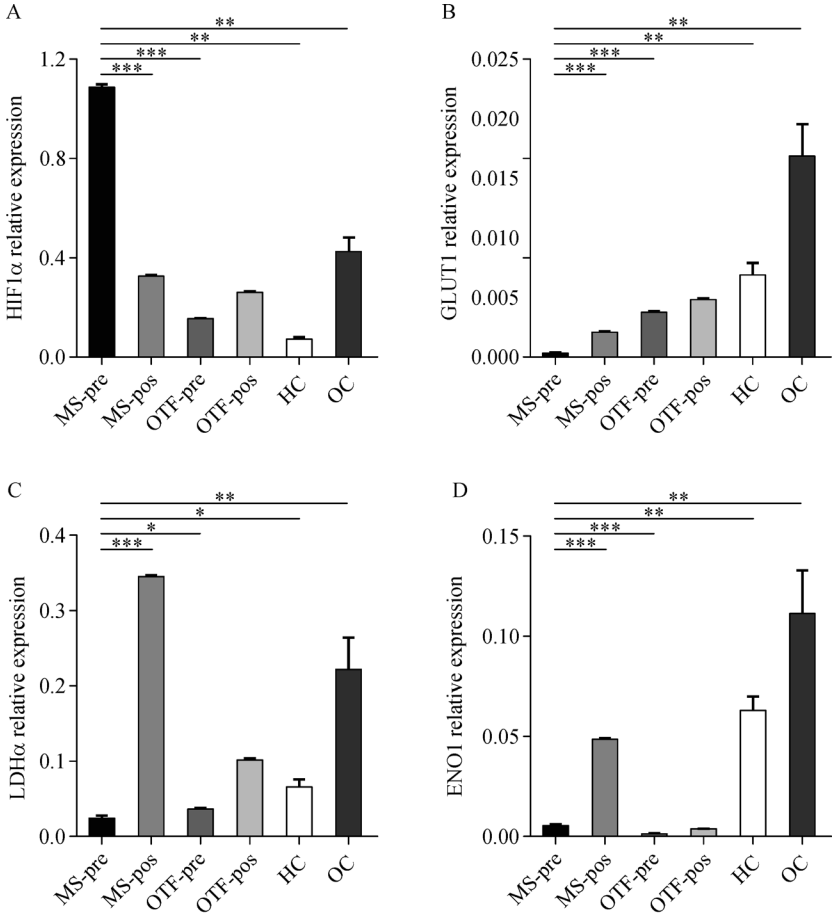

Fig. 4 Gene expression of glucose metabolism genes in peripheral $CD4^+$ T cells in a patient with MS. (A–D) Preoperative and postoperative gene expression levels of HIF1α, GLUT1, LDHα, and ENO1 in peripheral $CD4^+$ T cells in a patient with MS and a patient with OTF, patients with OC ($n = 9$) before the operation, and controls ($n = 10$). Data are shown as mean ± SEM of the three independent experiments. *$P < 0.05$, **$P < 0.01$, and *** $P < 0.001$.

manifestations, imaging findings (e.g., ultrasound, CT scanning, and MRI), and pathological characteristics have been widely used in describing MS [8]. However, the clinical laboratory features of patients with MS have not been thoroughly investigated to date. These features may be helpful in understanding and diagnosing MS.

CA125 is a tumor marker that is increased in most epithelial OCs, and its increase has been observed in other malignancies and benign tumors. Serum CA125 levels increase in patients with MS is caused by the mesothelial expression of CA125 rather than by the fibroma itself [9]. Peritoneal inflammation other than leiomyoma also leads to increased CA125 levels as verified by CA125 protein levels via immunohistochemistry [10]. However, the association between serum CA125 levels and amount of ascites relative to a low morbidity rate is controversial [11]. In the present study, serum CA125 and HE4 levels in patients with MS were significantly higher than those in patients with OTF and the controls. The serum CA125 and HE4 levels were significantly positively correlated with the presence of ascites. This finding on CA125 was consistent with findings of other studies that showed a relationship between increased serum CA125 levels and amounts of ascites [12]. However, the data on the association of increased HE4 levels and ascites in patients with MS are limited [13], as indicated in our study. Therefore, our findings have added to the knowledge of the features of the tumor markers CA125 and HE4 in patients with MS.

The recommended treatment for ovarian fibromas is surgical removal, but laparoscopic surgical management is reluctantly used because of difficultly in resecting complicated abdominal tumors. Ovarian fibroma is often misdiagnosed as uterine myoma and even mistakenly diagnosed as an ovarian malignant tumor because of the absence of characteristic clinical symptoms and imaging appearances. In our study, all the nine patients with MS underwent laparotomy, and more than half (55.56%) of the patients with OTF underwent laparoscopic surgery; these finding are consistent with the findings of a previous study [14]. The development of laparoscopic surgery in the fields of gynecology and obstetrics has led to advantages of less trauma, light pain, and rapid recovery. Ovarian fibroma can be removed from the abdominal cavity safely by laparoscopic surgery, with short hospitalization times and fast recovery [15,16]. Accumulating evidence suggests an increasing role of laparoscopic surgery, even robotic-assisted laparoscopy in oncological surgery for endometrial cancer, cervical cancer, and OC [17]. Therefore, the feasibility of laparoscopic surgery should be considered when MS diagnosis is uncertain.

Circulating immune-inflammatory cells, including lymphocytes, monocytes, neutrophils, and platelets derived from peripheral blood, not only affect the host immune system but also play a prominent role in antitumor immunity or tumorigenesis [18,19]. Several novel systemic inflammatory indicators, such as the NLR, PLR, LMR, and SII, reflect the systemic inflammatory and immune responses on the basis of the combination of lymphocytes, monocytes, neutrophils, and platelets. These indicators are calculated according to basic hematological parameters, which are easily obtained, low cost, well received, and widely used. These inflammatory indicators have strong associations with infectious diseases, autoimmune diseases, and even tumors [20,21]. Therefore, these inflammatory indicators are promising prognostic predictive factors for diseases, such as hepatocellular carcinoma, lung cancer, prostate cancer, and gastric cancer [22,23]. However, changes in these indicators in patients with MS remain poorly understood. In the current study, change in lymphocytes was most significant in peripheral blood cells regardless of the absolute counts or percentages of lymphocytes. Our results showed that the absolute count of lymphocytes and the percentage of lymphocytes were lower in the MS group than in the OTF and control groups, while the two lymphocyte measures were all significantly higher than in the OC group. These changes in lymphocytes led to a lower NLR and higher LMR in the MS group than in the OC group, and the NLR, PLR, and SII in the MS group were higher than in the OTF and control groups. Lymphocytes, as the key components of the immune cells, exert a crucial antitumor effect by secreting cytokines and inducing cytotoxic cell death. Few lymphocytes can directly reflect impaired immune surveillance, thereby leading to tumor cell proliferation, invasion, and migration. Therefore, relatively low number of lymphocytes may lead to an impaired peripheral immune response, thereby resulting in disorders in a host's peripheral immune system. Pleural effusion due to MS is related to inflammatory cytokines, including interleukin-6, interleukin-8, tumor necrosis factor, vascular endothelial growth factor, and fibroblast growth factor [24,25]. Neutrophils and platelets can facilitate the invasion, migration, and metastasis of tumor cells by inducing the secretion of the vascular endothelial growth factor and fibroblast growth factor. Therefore, the present study strongly suggests the involvement of circulating immune-inflammatory cells in the pathogenesis of MS, and the combination of these systemic inflammatory indicators may contribute to MS diagnosis.

The vital contribution of the immune surveillance system to tumor progression has been established. Our data showed that lymphocytes are the most significantly altered cells. Functional $CD4^+$ T cells, which are a major component of lymphocytes, preferentially reprogram their cellular metabolism under the pressure of tumor cells [26,27]. Therefore, we evaluated the expression levels of several glucose metabolism genes (HIF1α, GLUT1, ENO1, and LDHα) in peripheral $CD4^+$ T cells to identify specific molecular markers for the accurate diagnosis of MS. HIF1α is important to the determination of the fate of

T cells because it regulates cellular metabolism. The stimulation of HIF1α under hypoxia induces the expression of GLUT1 [27,28] and several downstream key enzymes encompassing the glucose metabolism pathway, including LDHα and ENO1 [29,30]. Therefore, we focused on the four glucose metabolism genes in the present study. We found that the peroperative HIF1α mRNA expression in the patient with MS was significantly higher than the HIF1α mRNA expression levels in patient with OTF, patients with OC, and controls. This finding suggested a hypoxic environment of peripheral $CD4^+$ T cells in a patient with MS before operation, whereas HIF1α was downregulated after tumor resection by surgery. However, GLUT1, LDHα, and ENO1 mRNA expression was repressed and then upregulated after tumor removal in the patient with MS, and thus glucose uptake and the glycolysis pathway of peripheral $CD4^+$ T cells in a patient with MS were weakened. The T cells' fate and immune responses are affected by the tumor microenvironment for altering T-cell cellular metabolic processes [31]. Activated T lymphocytes undergo a metabolic switch similar to tumor cells and upregulate aerobic glycolysis, thereby enabling proliferation and differentiation into specialized effector T cells [32]. Given the similarities of the tumor cells and T cells in metabolic profiles and nutrient requirements, the abnormally high metabolic rates and consumption of nutrients by tumor cells might compete with neighboring T cells [33] and may thus lead to T-cell metabolic exhaustion that underlies their functional exhaustion. Therefore, the microenvironment of the patients with MS may affect $CD4^+$ T-cell differentiation and proliferation by regulating the glucose metabolism pathway. Our results indicated that $CD4^+$ T cells cannot consume glucose as an energy source, and thus their growth and proliferation are reduced and immune dysfunction occurs. Consequently, our study showed an aberrant glucose metabolic process in the peripheral $CD4^+$ T cells of patients with MS. Our findings provided not only a solid foundation for the further study of MS but also evidence of differential diagnosis between MS and other ovarian tumors.

This study has several limitations, such as the few cases of MS and limited cell numbers. We detected the transcriptional levels of glucose metabolism genes in only one patient with MS and one patient with OTF. Further studies are required to determine whether specific genes can be used as diagnostic markers for MS. We were unable to detect the expression levels of glucose metabolism genes in $CD8^+$ T cells and regulatory T cells because of the insufficient number of cells. The mechanism involved in the impairment of HIF1α-mediated GLUT1 induction and resulting in the functional disorder of $CD4^+$ T cells requires further study.

In summary, our findings highlighted the clinical laboratory features and impaired glucose metabolism pathway in the peripheral $CD4^+$ T cells obtained from patients with MS. Understanding the metabolic regulation of peripheral $CD4^+$ T cells may identify potential markers for the early diagnosis of MS and provide a new way of studying the immunological characteristics of the disease in the future.

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Compliance with ethics guidelines

Wenwen Shang, Lei Wu, Rui Xu, Xian Chen, Shasha Yao, Peijun Huang, and Fang Wang declare that they have no conflict of interest. All included procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the *Helsinki Declaration*. Informed consent was obtained from all patients upon enrollment in the study.

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