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RESEARCH ARTICLE

Circulating myeloid-derived suppressors cells correlate with clinicopathological characteristics and outcomes undergoing neoadjuvant chemoimmunotherapy in non-small cell lung cancer

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Abstract

Purpose Myeloid-derived suppressors cells (MDSCs) are heterogeneous immunosuppressive cells, closely related to the development, efficacy and prognosis in various tumors. The relationship between clinicopathological characteristics, efficacy of neoadjuvant chemoimmunotherapy (NCIO) and circulating MDSCs in patients with non-small cell lung cancer (NSCLC) was investigated in this study.

Methods This study analyzed the clinical data of patients diagnosed at Department of Thoracic Surgery, Beijing Chest Hospital from November 2020 to August 2021. MDSCs and T cells subgroups were measured in fresh peripheral blood mononuclear cells(PBMCs) at baseline. Flow cytometry was used to detect MDSCs and T cells subgroups.

Results A total of 78 patients with NSCLC and 20 patients with benign nodule underwent direct surgery. 23 patients with NSCLC scheduled to accept NCIO before surgery. NSCLC had elevated levels of total MDSCs, PMN-MDSCs and M-MDSCs compared to patients with benign nodule. MDSCs subgroups were correlated to the pTNM stage in NSCLC patients. The frequency of total MDSCs were moderately positively correlated with regulatory T cells (Tregs)($r = 0.3597$, $P < 0.01$) and negatively correlated with CD4 + T cells($r = 0.2714$, $P < 0.05$). The baseline levels of total MDSCs, PMN-MDSCs and Tregs in pCR patients were significantly decreased than those of non-pCR patients ($P < 0.05$).

Conclusion Circulating MDSCs were increased in NSCLC patients. MDSC subgroups were related to pTNM stage in NSCLC patients. Total MDSCs were positively correlated with Tregs levels and negatively correlated with CD4 + T cells in peripheral blood. The level of MDSCs and Tregs in peripheral blood may have potential value in predicting pathological response in NSCLC.

Keywords MDSCs · NSCLC · Neoadjuvant · pCR

Introduction

Lung cancer (LC), with the highest cancer-related mortality, is one of the most common malignant tumors in the world [1]. Non-small cell lung cancer (NSCLC) accounts for about 80–85% of the newly diagnosed LC cases each year [2]. Surgical resection is still the main therapy for NSCLC. But 5-year survival rate is still unsatisfying for 60% in IIA and 36% in IIIA, respectively, because of recurrence and metastasis [3].

Neoadjuvant therapy is a promising treatment strategy to improve the long-term survival rate and cure rate of cancer patients, including chemotherapy, tyrosine kinase inhibitors (TKIs) and radiotherapy in single or multiple combinations [4]. In recent years, the emergence of immune checkpoint inhibitors(ICIs) has changed the treatment strategies of many advanced solid tumors, including NSCLC. ICIs in neoadjuvant therapy, blocking the inhibitory signal on the surface of cancer cells and re-activate the antitumor response by activating T cells, can promote early anti-tumor response and immune memory for long-term protective effect [5].

Several studies have shown that immunotherapy with ICIs gives satisfying effect on advanced NSCLC, which provides

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a new idea for neoadjuvant therapy of resectable NSCLC. Recently, neoadjuvant chemoimmunotherapy (NCIO) with PD-1 inhibitor shows a powerful efficacy for NSCLC in many clinical trials such as NCT03081689 [6]. However, about only 50% major pathological response (MPR)rate and lower complete pathological response (pCR) rate were reported in NCIO after surgery. Therefore, biomarkers that predict therapeutic response are essential for determining whether a patient is suitable for NCIO or to avoid unnecessary surgery after NCIO.

Myeloid-derived suppressor cells (MDSCs), composed of monocytes and granulocytes precursors, are phenotypically diverse population of bone marrow-derived cells. As one of components in tumor microenvironment(TME), these group of cells mediate their potent immunosuppressive effects, including facilitating escape from immune surveillance, mainly by suppressing T cell activation and proliferation [6]. MDSCs in peripheral blood have been demonstrated to correlate with tumor burden, stage, treatment response and outcomes in variety of cancers [7–9]. Especially, multiple studies have shown that high level of MDSCs indicate poor outcomes of ICIs [10, 11].

In this study, we aimed to analyze distribution of circulating immune cells populations including MDSC and T cell subgroups in NSCLC patients, and investigated their potential value in predicting outcomes for patients undergoing NCIO.

Materials and method

Patients and samples

We prospectively enrolled patients with pulmonary shadow in Beijing Chest Hospital from November 2020 to August 2021. All patients were over 18 years old and did not receive any forms of anti-tumor treatment. Blood samples were taken at baseline. Laboratory assay was conducted within 4 h. Patients for direct surgery obtained final pathological diagnosis. pTNM stage was reviewed by pathologists according to the 8th Edition Lung Cancer Staging System of the Joint Committee on Cancer of the United States.

NSCLC patients with NCIO schedule received needle biopsy or bronchoscopy to get the pathological diagnosis. The clinical stage was evaluated by senior physicians after positron emission tomography/computed tomography (PET/CT) examination. The inclusion criteria were as follows: (1) over 18 years old; (2) histologically diagnosed as resectable NSCLC (IA to IIIB, eighth edition of the American Joint Committee on Cancer); (3) primary tumor diameter $\geq$ 2 cm; (4) Eastern Cooperative Oncology Group (ECOG) score of 0. The exclusion criteria were as follows: (1) patients with druggable driver mutations/translocations; (2) history of any previous antitumor treatment; (3) suffering from known or suspected autoimmune diseases; (4) allergic to any component of monoclonal antibodies. According to international consensus, patients would undergo surgery within 2 weeks after NCIO which include 2–4 cycles of PD-1 inhibitors and chemotherapy (platinum and paclitaxel for squamous cell carcinoma, platinum and paclitaxel for adenocarcinoma). Response to NCIO were evaluated according to the Solid Tumor Response Evaluation Criteria (RECIST 1.1) before surgery, and pathological response were reviewed after surgery. All procedures involving human participants were in compliance with the Declaration of Helsinki (revised in 2013). The Ethics Committee of the Beijing Chest Hospital approved this study.

PBMCs isolation and flow cytometric analysis

The blood samples from patients were collected in the EDTA-K3 tube. PBMCs from fresh blood samples were isolated by Ficoll separation (Cytiva) within 4 h. PBMCs were counting in 1*10^6 cells and FC receptor blocker were added before stained with mAb, then analyzed by flow cytometry. MDSCs subgroups were identified referring to the previous literature and consensus [12]. Total MDSCs were defined as CD33 + /HLA-DR-, polymorphonuclear MDSCs(PMN-MDSCs) were defined as CD33 + /CD66 + /CD14-/HLA-DR-, and mononuclear MDSCs(M-MDSCs) were defined as CD33 + /CD14 + /CD66-/HLA-DR-(Fig. 1A). CD4 + T cells were defined as CD3 + /CD4 + , regulatory T cells(Tregs) were defined as CD4 + /CD25 + /CD127dim, and CD8 + T cells were defined as CD3 + /CD8 + (Fig. 1B).

Monoclonal antibodies for staining

The antibody concentration used in flow cytometry was optimized by single staining of PBMCs. All antibodies were listed below.

MDSCs: PerCP-CY 5.5 mouse anti-human CD45(Cytek bioscience), Alexa Fluor 647 mouse anti-human CD33(Biolegend), PE mouse anti-human CD66b(Biolegend), Brilliant Violet 605 mouse anti-human CD14(Biolegend), PE/Cyanine7 mouse anti-human HLA-DR(Biolegend),

T cells: Alexa Fluor 647 mouse anti-human CD25(Biolegend), FITC mouse anti-human CD3(Cytek bioscience), PE mouse anti-human CD4(Biolegend), PE/Cyanine7 mouse anti-human CD8(Biolegend), Brilliant Violet 421 mouse anti-human CD127(Biolegend).

Pathological assessment

Pathological response was evaluated by pathologists from Beijing Chest Hospital, Capital Medical University. The

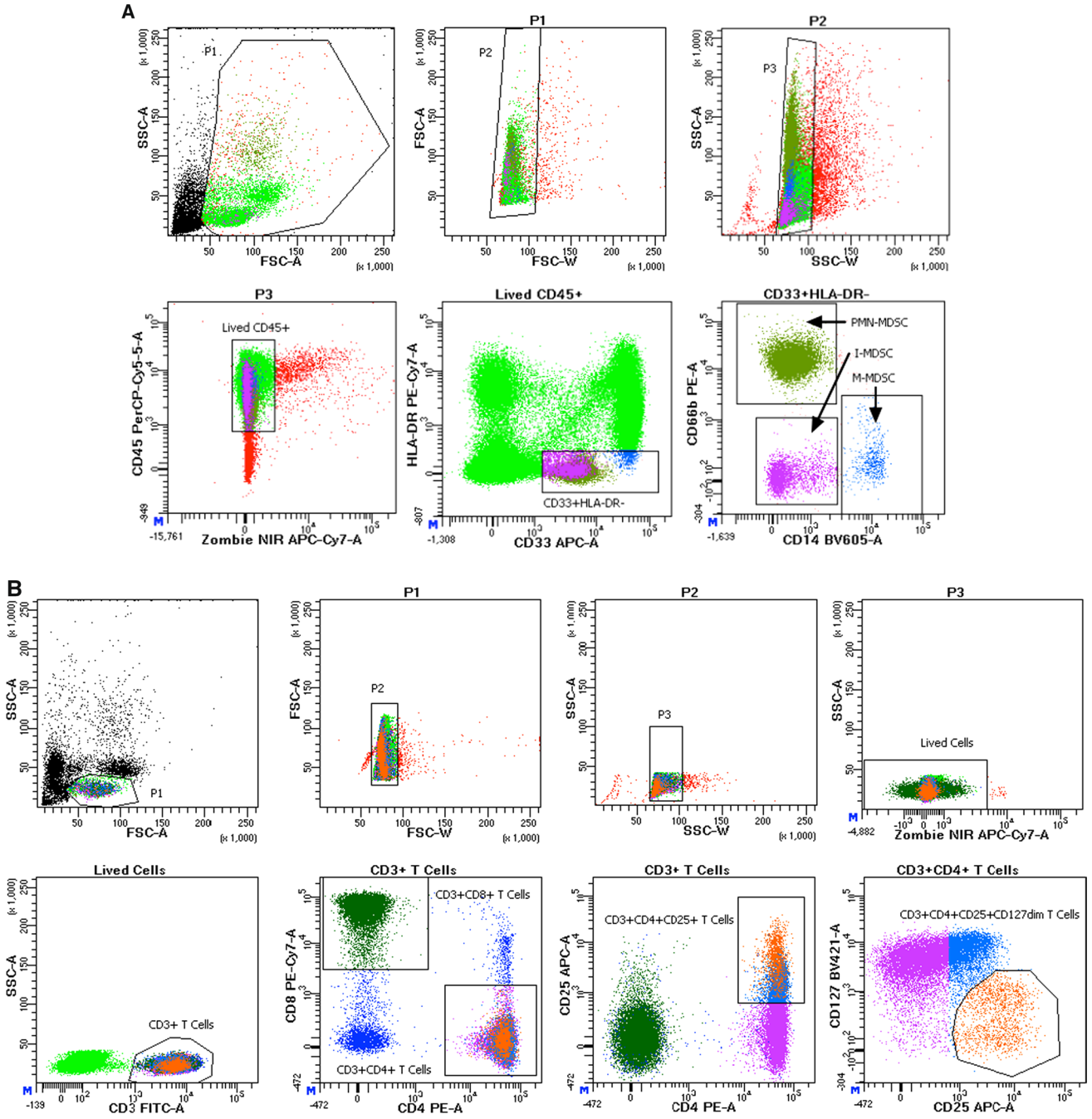


Fig. 1 **A** Flow cytometry gating strategy for MDSCs subgroups from patients. **B** Flow cytometry gating strategy for T cells subgoups from patients

method previously reported was used to measure the percentage of residual viable tumor cells from sectioned tissue samples in this study [13, 14]. Tumors with < 10% of viable tumor cells were defined as MPR, and those with no viable tumor cells were considered to be pCR.

Statistical analysis

Statistical analysis were performed by the GraphPad Prism 9.0 software package (GraphPad Software, Inc.). Data are presented as the median with interquartile

Table 1 Characteristic of NSCLC patients ($n = 78$)

Parameter	Number(%)
Age	
≤60	32 (41%)
>60	46 (59%)
Gender	
Male	45 (58%)
Female	33 (42%)
Smoking history	
No	44 (56%)
Yes	34 (44%)
Histological tumor type	
Squamous cell carcinoma	31 (40%)
Adenocarcinoma	47 (60%)
pTNM stage	
I-II	48 (62%)
III	30 (38%)
pT stage	
T1-T2	53 (68%)
T3-T4	25 (32%)
pN stage	
N0	43 (55%)
N1-N2	35 (45%)

range. The Mann–Whitney U test was used to compare the differences in the baseline levels of immune cells between two groups. The correlation between circulating MDSCs and T cells were assessed by Pearson's correlation test. The correlation between characteristic of patients and pathological response were assessed by Fisher's exact test. $P < 0.05$ was considered to be statistically significant.

Results

Patients characteristics

98 patients for directly operation were analyzed in this study. After surgery and pathological examination, 78 patients were confirmed as NSCLC, including 31 Squamous cell carcinoma and 47 Adenocarcinoma. 20 patients were confirmed as benign nodules. The patients with NSCLC and benign nodules included 45 and 12 males as well as 33 and 8 females with median age of 57.5 and 61, respectively. 48 patients with NSCLC were in early to mid-stage (pI–pII) and 24 patients were in advanced stage (pIII) (Table 1).

Frequency of MDSC subgroups were increased in NSCLC patients

All blood samples were collected before surgery and PBMCs of those were purified within 4 h. Frequency was defined as the number of MDSCs in live CD45 + cells. Flow cytometric analysis showed that total MDSCs, PMN-MDSCs and M-MDSCs were significantly increased in patients with NSCLC compared to those in patients with benign nodules($P < 0.05$). However, there was no difference in I-MDSCs between patients with NSCLC and benign nodules(Fig. 2).

Frequency of MDSC subgroups correlate with clinicopathological features in patients with NSCLC

To detect the distribution of MDSCs subgroups in patients with NSCLC, we compared frequency of those in different clinicopathological characteristics, including age, gender, histological subtypes and tumor stage. We observed that

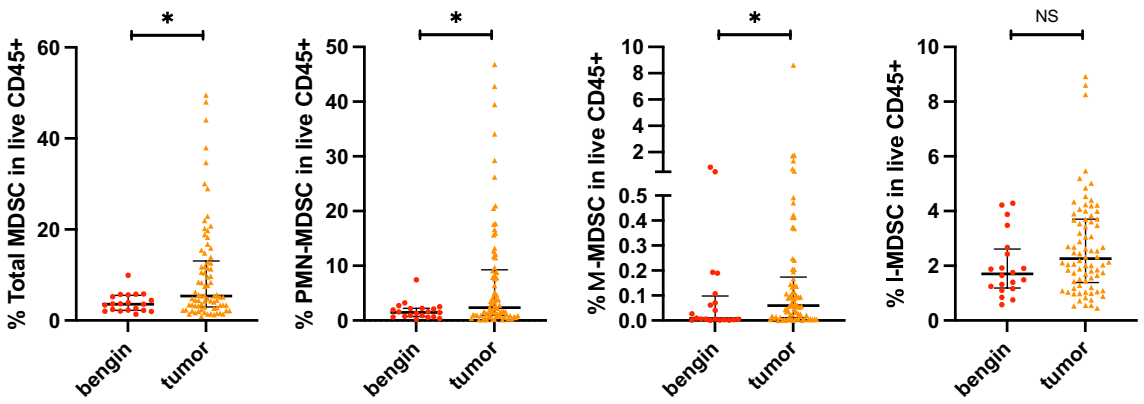


Fig. 2 Frequency of MDSCs from 78 patients with NSCLC and 20 patients with lung bengin nodole. Data are presented as median ± interquartile range,*$P < 0.05$. *NS* not significant

there was no significant difference in MDSCs subgroups among different histological subtypes, age and genders. Total MDSCs, PMN-MDSCs and I-MDSCs were expanded in patients with advanced stage ($P<0.001$). Rising trend was found in patients with stage III but there was no significantly difference ($P=0.09$). Compared with patients with smaller tumor size (T1–T2), total MDSCs and its three subgroups (PMN-MDSC, M-MDSCs and I-MDSCs) were significantly increased in patients with larger tumor size (T3–T4) ($P<0.01$, $P<0.01$, $P<0.05$ and $P<0.01$, respectively). Similarly, compared with NSCLC patients without lymph node metastasis (N0), MDSCs and three subgroups were significantly increased in patients with lymph node metastasis (N1-N2) ($P<0.001$, $P<0.001$, $P<0.05$ and $P<0.01$, respectively) (Fig. 3A).

The frequency of Tregs in patients with advanced NSCLC were significantly increased ($P<0.001$). Compared with NSCLC patients with smaller tumors (T1–T2), Tregs were significantly increased in patients with larger tumor size ($P<0.001$). Compared with NSCLC patients without lymph node metastasis (N1), Tregs were significantly increased in patients with lymph node metastasis (N1–N2) ($P<0.01$) (Fig. 3B).

Correlation between MDSC frequency and T cell subgroups in patients with NSCLC

MDSCs with the function of suppressing T cells also inhibit immune response in vivo by mediating Treg induction [15]. We investigated the correlation between MDSC and T cell subgroups in peripheral blood of NSCLC patients, including CD4 + T cells, CD8 + T cells and Treg cells. In NSCLC patients, the total MDSC frequency and the percentage of CD4 + T cells showed a low degree of negative correlation ($r=-0.2714$, $P<0.05$). The total MDSC frequency was moderately positively correlated with the percentage of Tregs ($r=0.3597$, $P<0.01$). However, the total MDSC frequency and CD8 + T cells showed a positive correlation, which was inconsistent with the expected results ($r=0.3014$, $P<0.01$) (Fig. 4).

Efficacy of NCIO in patients with NSCLC

From November 2020 to August 2021, 23 patients scheduled to receive NCIO and underwent surgery in total. The detail of those are shown in Table 2, including 20 males and 3 females. After biopsy by needle or bronchoscopy, 17 of squamous cell carcinoma and 6 of adenocarcinoma in patients were diagnosed. All patients receiving NCIO had no druggable driver mutations/translocations. 1 patient was in stage I, 3 patients were in stage II, and 19 patients were in stage III. Among patients with NCIO, 3 of those received thoracotomy; 20 of those received video-assisted thoracic surgery(VATS). 20 of those underwent lobectomy; 2 of those underwent sleeve resection, and 1 of those received pneumonectomy. R0 resection was achieved in all patients. The average blood loss was 202 ml (50 ml–800 ml), and the average operation time was 174 min (85–290 min).

After the completion of NCIO, the tumor remission was evaluated according to RECIST 1.1. 16 of patients were assessed for partial remission(PR), and 7 patients were assessed for stable disease (SD). After surgical resection, pathological response was evaluated by professional pathologist. In the end, the pathology results showed MPR in three of patients and nine patients achieved pCR (Table 3). In the analysis of the correlation between pathological response and characteristic in patients with NCIO, we found that patients in early to mid-stage (cI–cII) or with no lymph node metastasis were more likely to achieve pCR ($P<0.05$). PD-L1 expression was not related to pCR or MPR ($P>0.05$) (Supplemental Table 1) (Supplemental Table 2).

MDSCs and Tregs frequency are associated with pathological response

MDSCs and Tregs are immunosuppressive cells in vivo. We guessed that the level of MDSCs and Tregs at baseline were correlated with efficacy of NCIO in patients with NSCLC. The frequency of total MDSCs and PMN-MDSCs in patients with pCR was significant increased compared with those with non-pCR. Compared with non-MPR patients, the frequency of total MDSCs and PMN-MDSCs in patients that achieved MPR including pCR also got a rising trend, but there was no statistical difference ($P=0.08$ and $P=0.06$) (Fig. 5).

The frequency of Tregs in non-pCR patients was significantly higher than that in pCR patients ($P<0.05$). The frequency of Tregs in patients that achieved MPR including pCR was also significantly higher than that of MPR patients ($P<0.05$). However, CD4 + T cells and CD8 + T cells did not show significant difference (Fig. 6).

We also analyzed the relation of immune cells to remission according to RICIST 1.1. However, the frequency in two types of immunosuppressive cells between SD and PR patients did not show significant difference similar to pathological response ($P>0.05$) (Supplemental Fig. 1).

Discussion

MDSCs, inhibit the immune response of T cells through the production of reactive oxygen species or nitrogen oxides, are one of the components in TME [7]. Several studies have

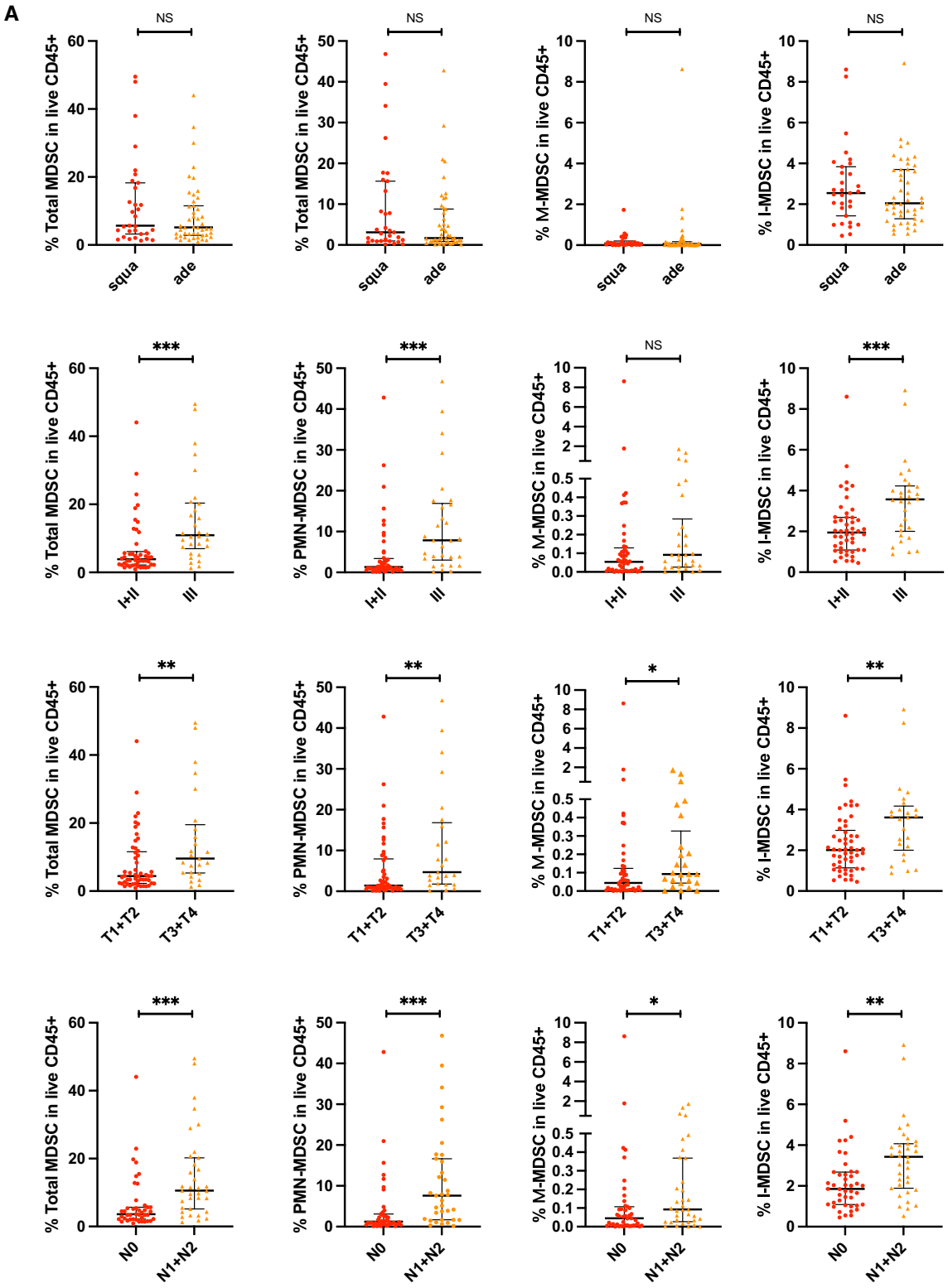


Fig. 3 **A** Analysis of MDSCs frequency from 78 NSCLC patients in histological subtypes and pathological stage. Data are presented as median ± interquartile range, $*P<0.05$, $**P<0.01$, $***P<0.001$. NS, not significant. **B** Analysis of T cells subgroups from 78 NSCLC patients in pathological stage. Data are presented as median ± interquartile range, $**P<0.01$, $***P<0.001$. *NS* not significant

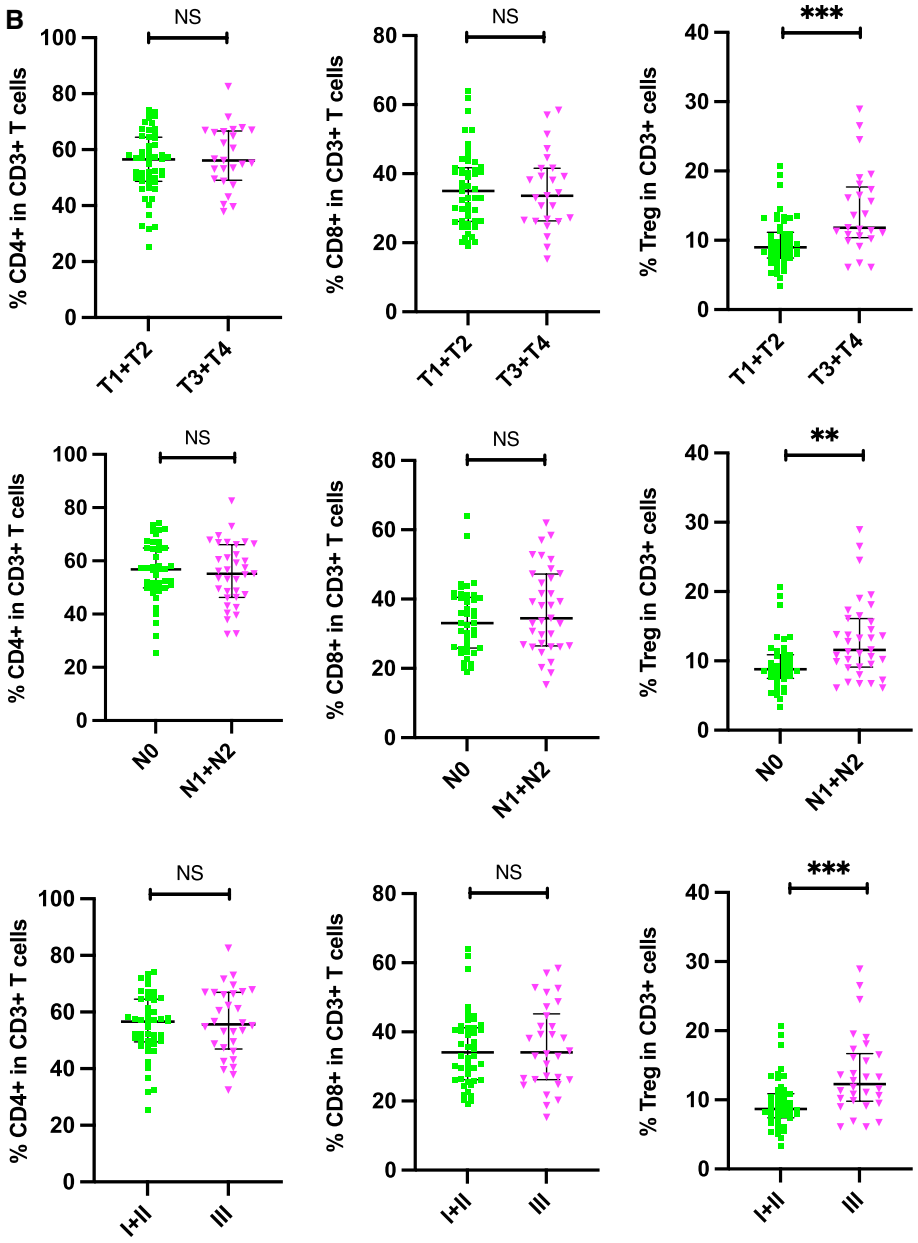


Fig. 3 (continued)

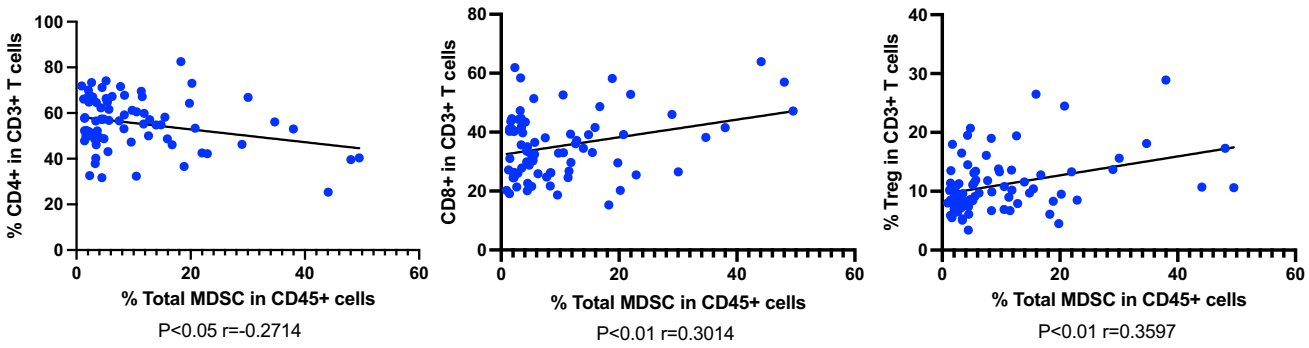


Fig. 4 The correlation of total MDSCs frequency with T cells subgroups

Table 2 Characteristic of NSCLC patients with NCIO ($n=23$)

Parameter	Number(%)
Age	
≤60	8 (35%)
>60	15 (65%)
Gender	
Male	20 (87%)
Female	3 (13%)
Smoking history	
No	7 (30%)
Yes	16 (70%)
Histological tumor type	
Squamous cell carcinoma	17 (74%)
Adenocarcinoma	6 (26%)
cTNM stage	
I	1 (4%)
II	3 (13%)
III	19 (83%)
cT stage	
T1	2 (9%)
T2	9 (39%)
T3	5 (22%)
T4	7 (30%)
cN stage	
N0	10 (43%)
N1	3 (13%)
N2	10 (43%)
PD-L1 expression	
Negative	3 (13%)
Positive	11 (48%)
Missing	9 (39%)

Table 3 Details of NSCLC patients undergo NCIO in perioperative period

Factor	Results	Numbers(%)
Cycles	2	11(48%)
	3	12(52%)
Surgical approach	Open	3(13%)
	VATS	20(87%)
Scope of resection	Lobectomy	20(87%)
	Sleeve resection	2(8%)
	Pneumonectomy	1(4%)
Surgical margin	R0	23(100%)
	R1	0(%)
Pathological response	<MPR	11(48%)
	MPR	3(13%)
	CPR	9(39%)
RECIST status	SD	7(30%)
	PR	16(70%)
Operation time[a]	174 min(85-290 min)	
Bleeding[a]	202 ml(50 ml-800 ml)	
Drainage volume[a]	1368 ml(100-3040 ml)	

[a]Presented as mean (range)

shown that tumor cells induce MDSCs, and at the same time, MDSCs endow tumor cells with the ability to proliferate, migrate and invade [7, 16]. MDSCs can be enriched in the peripheral blood of patients with multiple types of cancer, closely related to clinical stage and overall survival, indicating that circulating MDSCs play an important role in tumor progression [7, 17, 18]. In our study, we also found that circulating MDSCs were correlated with clinicopathological characteristics of patients with NSCLC. The results demonstrated that the frequency of MDSC subgroups were significantly increased in NSCLC patients. The expansion of MDSCs were related to the pathological stage, tumor size and lymph node metastasis status. Therefore, MDSCs may be one of the factors for progression in NSCLC. MDSCs mediate immune suppression by Treg cells induction [15] We also explored the correlation between MDSC and T cell subgroups in NSCLC patients. Our results showed that MDSCs and Tregs were moderately positively correlated, which suggested that MDSC-induced Tregs proliferation might be one of the reasons for the immunosuppressive status of NSCLC patients. However, our results showed a positive correlation between the frequency of total MDSCs and CD8 + T cells. We guessed that this phenomenon might associate with the decreased of total CD3 + T cells or some mechanism in negative feedback in vivo.

NSCLC accounts for approximately 85% and is the most common type of lung cancer. Among them, about 40% of patients have the opportunity for surgery in stage I–III at the first diagnosis [19]. However, several patients undergoing radical surgical resection get poor survival outcomes due to cancer recurrence. For NSCLC patients in stage I–II, the recurrence rate is about 20% after surgical resection, while for patients in stage IIIA, the recurrence rate is as high as 40% [20].

Potential benefits have been proven in neoadjuvant immunotherapy before surgical resection. First of all, due to original tumor burden in body before surgery, T cells can be activated maximumly by the exposure of tumor antigens and cause more lasting immune effects for reducing recurrence [5]. Second, neoadjuvant therapy eliminates potential micro-metastasis, reducing the risk of recurrence and metastasis after surgery. Neoadjuvant therapy also reduces tumor stage and promotes the possibility of complete resection, especially for patients with large tumors or tumors in anatomical locations where it is difficult to be resected [21]. Immunotherapy combined with chemotherapy is confirmed to be more reasonable in several types of cancer. Several studies have shown that chemotherapy can inhibit

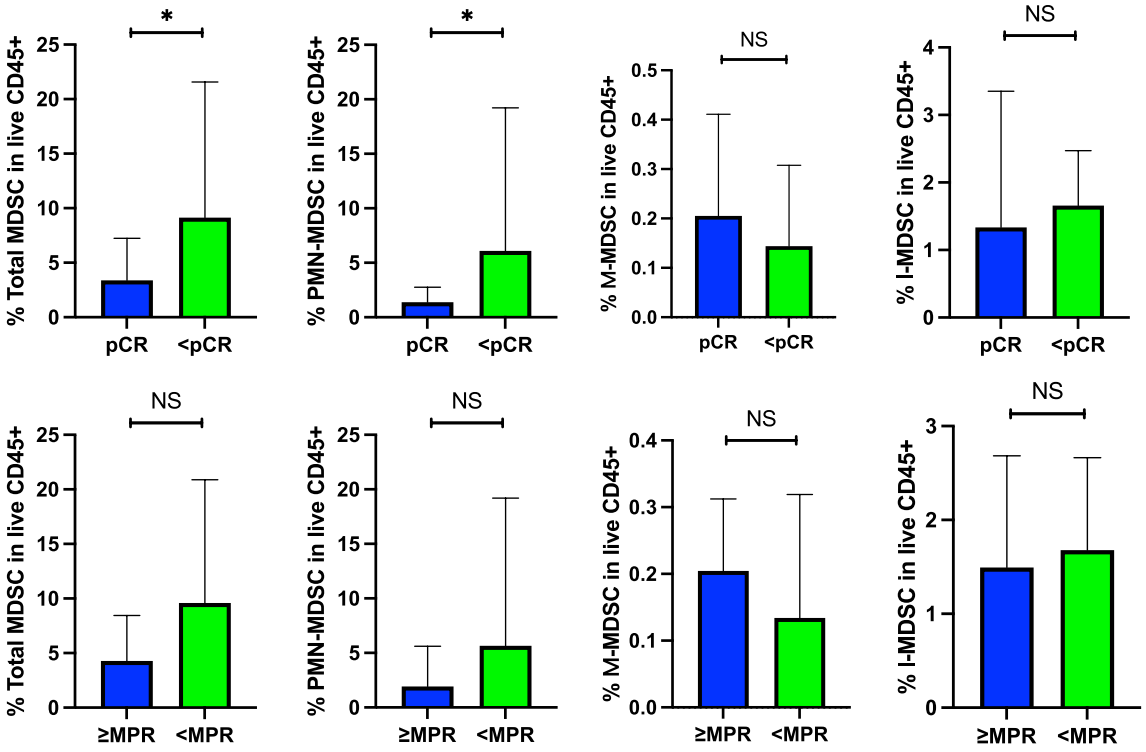
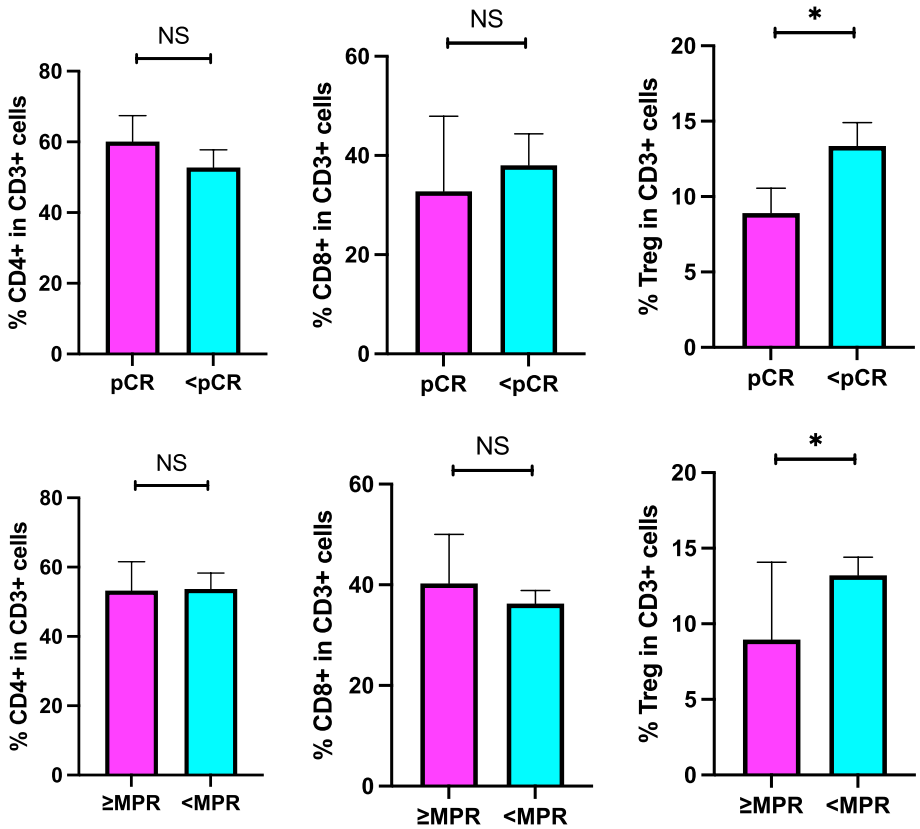


Fig. 5 Frequency of MDSCs in patients with different pathological response. Data are presented as median ± interquartile range, $*P < 0.05$. *NS* not significant

Fig. 6 Frequency of T cells subgroups in patients with different pathological response. Data are presented as median ± interquartile range, $*P < 0.05$. *NS* not significant



immunosuppressive cells, promote the immunogenicity of tumor and increase T lymphocyte infiltration, thereby transforming "cold tumors" into "hot tumors" [22].

At present, a number of clinical studies have confirmed the efficay of NCIO. The results of the NADIM trial (NCT03081689) showed that the MPR rate of NCIO with three cycles of nivolumab combined with carboplatin and paclitaxel was as high as 85%, of which 71% reached PCR and the R0 resection rate reached 100% [23]. Shu et al. reported the MPR rate of atezolizumab combined with chemotherapy for two cycles was 57%, and the pCR rate was 33% [14]. In our cohort, a total of 23 patients with NSCLC received NCIO and all of them got R0 resection. After professional pathological assessment, 12 (52.17%) of 23 people achieved MPR, and 9 (39.13%) achieved pCR. Patients in early to mid-stage (cI–cII) were more likely to achieve achieve pCR. Based on several articles [24, 25], we guessed that poor response of patients after NCIO might relate to impaired immune function due to their lymph node with tumor metastasis.

Partial patients with resectable NSCLC can be benefit from NCIO, so biomarkers to identify patients who are more possible to get response are essential for NCIO. PD-L1 and TMB have shown predictive efficacy on single immunotherapy or chemotherapy combined with immunotherapy for advanced NSCLC [26]. However, there is no consensus on evaluating the response of NCIO for now.

Local immune status can be reflected according to the characteristics of immune cells in tumor bed [27]. Recent studies have shown that T cells in PD-1 response are derived from the pool of T cells clones migrating from peripheral blood [28]. Therefore, immune cells in peripheral blood are potential to predict the therapeutic effect of immune checkpoint inhibitors such as anti-PD-1 earlier.

A large amount of evidences show that MDSCs inhibit the activity of immune cells, closely related to the therapeutic effect of immune checkpoint inhibitors [29, 30]. Tregs are also suppressive immune cells in peripheral blood. Their value as a predictive marker of ICI efficacy in cancer has been demonstrated in numbers of studies [31, 32].

In our study, we found that the total MDSCs, PMN-MDSCs, and Tregs from the peripheral blood at baseline between the pCR group and the non-pCR group were statistically different, and patients who did not achieve pCR were increased than those of pCR patients. At the same time, Tregs in the non-MPR group were also significantly higher than those in the MPR group. These evidences indicated that the immunosuppressive cells derived from peripheral blood were potential to predict the pathological response for patients undergoing NCIO, which might help avoid unnecessary surgical treatment.

There are still some limitations in this study. First of all, the cohort is not big enough. At the same time, the study does not strictly require patients in uniform PD-1 inhibitors and platinum drugs. Therefore, it is still necessary to expand the sample size and stricter enrollment requirements to confirm the reliability. Second, because the cohort has not yet reached the median time of patient recurrence-free survival, there is a lack of data on OS. Follow-up data are needed to determine whether NCIO can improve the PFS and OS of NSCLC patients, and whether peripheral blood immune cell characteristics including MDSCs have the potential to predict patient survival.

Conclusion

The increase of MDSCs were related to the pathological stage, tumor size and lymph node metastasis in NSCLC. MDSCs in peripheral blood of NSCLC patients were moderately positively correlated with Tregs and negatively correlated with CD4 + T cells. NCIO is effective for NSCLC patients. The distribution of Tregs and MDSCs in peripheral blood might be potential biomarker in predicting the pathological response of NCIO.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12094-021-02765-9.

Declarations

Conflict of interest All authors in this study declare no competing interests.

Ethical approval This research was approved by the Institutional Ethics Committee in Beijing chest hospital and was carried out according to the Ethical Principles of the Declaration of Helsinki.

Informed consent All patients provided written informed consent before study inclusion.

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