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## 1 **Inflammation-based scores in patients with pheochromocytoma**

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14  
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## 8 **Abstract**

9 **Background:** Pheochromocytoma is associated with systemic inflammation, but the underlying  
10 mechanisms are unclear. Therefore, we investigated the relationship between plasma metanephrine levels  
11 and haematological parameters – as a surrogate of inflammation – in patients with pheochromocytoma and  
12 the influence of preoperative  $\alpha$ -blockade treatment.

13 **Design and Methods:** We retrospectively studied 68 patients with pheochromocytoma who underwent  
14 adrenalectomy (median age 53 years, 64.7% females) and two control groups matched for age, sex, and  
15 body mass index (BMI): 68 patients with non-functioning adrenocortical tumors (NFAT) and 53 with  
16 essential hypertension (EAH). The complete blood count (CBC) and several inflammation-based scores  
17 [Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lymphocyte-to-Monocyte  
18 Ratio (LMR), Systemic-Immune-Inflammation Index (SII), Prognostic-Nutrition Index (PNI)] were  
19 assessed in all patients and, in a subset of pheochromocytomas, after adrenalectomy (n=26) and before and  
20 after preoperative  $\alpha$ -blockade treatment (n=29).

21 **Results:** A higher inflammatory state, as indicated by both CBC and inflammation-based scores, was  
22 observed in patients with pheochromocytoma compared to NFAT and EAH. Plasma metanephrine levels  
23 showed a positive correlation with NLR (r=0.4631), PLR (r=0.3174), SII (r=0.3709), and a negative  
24 correlation with LMR (r=0.4368) and PNI (r=0.3741), even after adjustment for age, sex, ethnicity, BMI

1 and tumor size (except for PLR). After adrenalectomy, we observed a reduction in NLR ( $p=0.001$ ), PLR  
2 ( $p=0.003$ ), SII ( $p=0.004$ ) and a concomitant increase in LMR ( $p=0.0002$ ). Similarly,  $\alpha$ -blockade treatment  
3 led to a reduction in NLR ( $p=0.007$ ) and SII ( $p=0.03$ ).

4 **Conclusions:** Inflammation-based scores in patients with pheochromocytoma showed pro-inflammatory  
5 changes that correlated with plasma metanephrine levels and are ameliorated by adrenalectomy and  $\alpha$ -  
6 blockade.

## 7

## 8 **Introduction**

9 Pheochromocytomas are rare neuroendocrine tumors arising from the chromaffin cells of the  
10 adrenal medulla that typically secrete excessive amounts of catecholamines (1). Chronic exposure to high  
11 levels of catecholamines is responsible for most of the clinical manifestations of pheochromocytoma,  
12 including the classic triad of headache, palpitations, and profuse sweating, as well as significant  
13 hemodynamic and metabolic changes.

14 Beyond their well-known effects on the cardiovascular system (2,3) and metabolism (4,5),  
15 catecholamines also influence the immune system. Previous studies have shown that catecholamines  
16 directly modulate innate immune cell function *in vitro* and *in vivo* (6–9) and regulate the production of  
17 pro-inflammatory cytokines (10). The effects of catecholamines on the immune system are mediated by  
18 the adrenergic receptors expressed by immune cells. The  $\beta_2$ -adrenoceptor is thought to be most involved  
19 in inflammatory processes, but increasing evidence suggests the role of other adrenergic receptors,  
20 particularly the  $\alpha_1$  subtype (11–13). So far, some evidence suggests that patients with pheochromocytoma  
21 may have an increase in several inflammatory markers, which recovers after the tumor removal (9,14,15).  
22 Interestingly, patients with pheochromocytomas and paragangliomas and an increased inflammatory state  
23 have been suggested to have a reduced survival (16).

1 Recently, several inflammation-based scores have been proposed as potential markers of systemic  
2 inflammation in several diseases, such as ischaemic heart disease, stroke, and cancer (17–20). The  
3 increasing interest in these markers is due to their recognized prognostic value as well as their cost-  
4 effectiveness, wide availability, and practicality. The combination of common serum-based parameters,  
5 such as complete blood count (CBC) and acute-phase proteins, can predict acute and chronic  
6 inflammation. An increase in the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio  
7 (PLR) and systemic immuno-inflammation index (SII, the product of platelet count and NLR), and a  
8 decrease in the lymphocyte-to-monocyte ratio (LMR) and prognostic nutrition index (PNI, that consider  
9 serum albumin and the absolute lymphocyte count) reflect ineffective immune surveillance or an increased  
10 inflammatory state (21).

11 Scarce data are available about the relationship between the inflammation-based scores and  
12 catecholamines secretion in pheochromocytomas and, in particular, about the effect of the normalization  
13 of catecholamine secretion. Moreover, the possible role of  $\alpha$ -blockers in modulating these parameters is  
14 unknown.

15 The aim of this study was, therefore, to evaluate: (i) the levels of several inflammation-based  
16 scores in patients with pheochromocytoma before and after adrenalectomy and their relationship to  
17 clinical characteristics and catecholamine levels; (ii) the possible impact of  $\alpha$ -blockers on the  
18 inflammation-based scores.

## 20 **Subjects and Methods**

### 21 *Patient cohort*

22 We performed a retrospective electronic clinical records review of 68 patients with  
23 pheochromocytoma, 68 patients with non-functioning adrenal tumors (NFAT), and 53 patients with  
24 essential hypertension (EAH).

1 Patients with pheochromocytoma were diagnosed between January 2001 and April 2023 and  
2 followed up in the Adrenal Tumor Service at the Queen Elizabeth Hospital Birmingham (UK). Only  
3 patients with pheochromocytomas who underwent surgery with subsequent normalization of  
4 catecholamine levels and with available clinical and biochemical data including CBC before and after  
5 adrenalectomy were included. In accordance with current guidelines (1), the diagnosis of  
6 pheochromocytoma was confirmed histologically or based on the combined presence of increased plasma  
7 metanephrines and detection of an indeterminate adrenal mass on imaging. If the exact values of  
8 metanephrines and normetanephrines were not available because they were initially diagnosed in another  
9 centre, the pheochromocytomas were considered hypersecretive based on the information in the referral  
10 letter. Patients with paragangliomas or metastatic pheochromocytomas were excluded. Patients with  
11 conditions that could significantly affect the CBC, such as infections, haematological diseases, severe  
12 cardiomyopathy, active malignancies, active autoimmune diseases, and treatment with oral  
13 glucocorticoids or other immunomodulatory drugs were also excluded (**Supplementary Figure 1**) (22).

14 Patients with NFAT or EAH matched for age, sex, and body mass index (BMI) to the cohort of  
15 pheochromocytoma, and with available CBC were used as control groups (**Supplementary Table 1**) (22).

16 NFAT were defined by the presence of adrenocortical adenomas and cortisol values after 1 mg-  
17 overnight dexamethasone suppression test <50 nmol/L (1.8 µg/dL) (23). Patients with NFAT were  
18 followed at the Adrenal Tumor Service of the Queen Elizabeth Hospital, Birmingham, UK (diagnosed  
19 between January 2001 and April 2023).

20 EAH were defined according to international guidelines (24), and without conditions associated  
21 with increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, including diabetes mellitus type  
22 2 (T2DM). Patients with any of the above conditions that could affect the CBC were excluded. Patients  
23 with EAH were followed at the Hypertension Centre of the Istituto Auxologico Italiano, Milan, Italy,  
24 between June 2019 and April 2023.

25 *Study Design*

1 Data were collected at the time of tumor diagnosis (*baseline*) and at three different time points  
2 after adrenalectomy: (i) at the last day of hospital admission (*immediately post-surgery*, n=68, median  
3 time 3 days, interquartile range, IQR 2-5); (ii) at least one month after and within one year of surgery  
4 (*short-term follow-up*, n=18, median time 5 months, IQR 1.8-7.9); (iii) at least 1 year after surgery (*long-*  
5 *term follow-up*, n=15, median time 39.6 months, IQR 26.4-55.2). In patients with bilateral metachronous  
6 tumors, defined as pheochromocytomas developing in a contralateral side after at least 6 months of the  
7 initial tumor (25), data before and after second surgery were considered. Control patients were assessed  
8 only once.

9 This study has been conducted in accordance with the Declaration of Helsinki. Institutional review  
10 board approval for retrospective data review from patients with pheochromocytoma undergoing routine  
11 clinical care was obtained from the University Hospital Birmingham NHS Foundation Trust (audit  
12 reference CARMS-18152). Ethical approval has been obtained for study research by both local institutions  
13 (NHS Health Research Authority - Prime-Act study IRAS 261291, RG 19-028, and Istituto Auxologico  
14 Italiano of Milan Code 2019\_01\_29\_06).

#### 15 *Data collection*

16 Demographic and clinical data were collected for all patients at the time of diagnosis, including  
17 the presence of adrenergic symptoms (i.e., palpitations, sweating, tremors, anxiety) as well as the  
18 cardiometabolic comorbidities typically associated with catecholamine excess, such as hypertension  
19 (HNT), cardio-cerebrovascular events (CVE), T2DM, and obesity (BMI >30 kg/m<sup>2</sup>). Additionally, details  
20 of antihypertensive treatment, including  $\alpha$ -blockade, were collected in patients with pheochromocytoma.  
21 The immediate preoperative  $\alpha$ -blocker dose was considered in the analysis. The biochemical evaluation  
22 included the determination of plasma metanephrines and normetanephrine (data available in 59 and 62  
23 patients, respectively), as well as CBC, serum albumin levels and C-reactive protein (CRP) levels when  
24 available (n=38, all measurements with values  $\leq 10$  mg/L). Plasma metanephrines and normetanephrine  
25 were measured by liquid chromatography-tandem mass spectrometry using the Chromsystems

1 MassChrom® Free Metanephrines in Plasma commercial kit. Plasma metanephrine and normetanephrine  
2 at the time of CBC sampling were used for the analysis.

3 Inflammation-based scores were calculated from serum albumin and CBC (**Supplementary Table**  
4 **2**) (17,21,22). NLR and PLR were calculated by dividing the absolute neutrophil or platelet counts,  
5 respectively, by the lymphocyte count. LMR is obtained by dividing the absolute lymphocyte count by the  
6 monocyte count, while the SII is the product of the absolute platelet count and NLR. The PNI reflects not  
7 only the inflammatory status but also the nutritional status of the patient and is obtained by multiplying  
8 serum albumin by 5 times the absolute lymphocyte count.

9 Radiological features of the adrenal mass, such as maximum diameter and side of the lesion, were  
10 also collected. For bilateral adrenal tumors, the diameter of the largest mass was considered.

11 Two different histopathological scores (Pheochromocytoma of the Adrenal Gland Scaled Score,  
12 PASS, and Grading system for Adrenal Pheochromocytoma and Paraganglioma, GAPP) were recorded  
13 (26) and details about postoperative complications and duration of hospital admission were specified. In  
14 addition, data about genetic screening were collected. Genetic testing for germline variants that predispose  
15 to pheochromocytoma was offered to all patients who met the national eligibility criteria (27). A targeted  
16 next-generation sequencing (NGS) gene panel was used for genetic testing, evaluating coding regions in  
17 *FH*, *MAX*, *MEN1*, *RET*, *SDHAF2*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *TMEM127*, and *VHL*. *NF1* was tested  
18 only in the presence of clinical features of neurofibromatosis type 1. Genetic data were not available for  
19 26 patients because (i) they did not meet eligibility criteria for genetic testing, (ii) they did not provide  
20 consent, or (iii) test results were pending at the time the study was conducted.

### 21 *Statistical analysis*

22 Descriptive statistics were expressed as numbers and percentages for categorical variables and as  
23 median and interquartile range (IQR) for continuous variables. Comparisons between patients with  
24 pheochromocytoma, NFAT, and EAH were performed using the Mann-Whitney U test and Kruskal-



1 Wallis test followed by Dunn's post hoc test. Analysis of the paired continuous values (data pre- and post-  
2 operative as well as before and after  $\alpha$ -blockade treatment) was performed using the Wilcoxon test.  
3 Spearman's correlation analysis was performed to evaluate the relationship between  
4 metanephrines/normetanephrine and inflammatory parameters in patients with pheochromocytoma before  
5 surgery. Uni- and multivariate linear regression were performed to confirm the association found between  
6 the metanephrine levels and inflammation-based scores and adjust for age, sex, ethnicity, BMI and tumor  
7 size of pheochromocytoma. In this analysis, all continuous variables were transformed to the natural  
8 logarithm of their value.

9 A p-value of  $<0.05$  was considered statistically significant. Statistical analysis was performed by  
10 GraphPad Prism version 9.

## 12 **Results**

### 13 *Patient characteristics at baseline*

14 A total of 68 patients with pheochromocytoma were included in the study. Clinical  
15 characteristics are shown in **Table 1**. The majority were females (n=44, 64.7%) of Caucasian ethnicity  
16 (n=46, 67.6%) with a median age at diagnosis of 53 years (IQR 41.3-69). Among patients who  
17 underwent genetic testing to assess the presence of germline mutations predisposing to  
18 pheochromocytoma, 33.3% of whom were found to have genetic defects, most commonly in the *RET*  
19 gene (n=6). The most common mode of presentation was detection during workup for an adrenal  
20 incidentaloma (n=32, 47.1%), followed by adrenergic symptoms (n=31, 45.6%), and detection during  
21 screening for a known underlying genetic susceptibility (n=5, 7.4%). Regarding the associated  
22 cardiometabolic morbidities, most patients were hypertensive (n=52, 76.5%), of whom 35.3% (n=24)  
23 were taking more than one antihypertensive medication; T2DM and obesity were present in 25% and  
24 30.9%, respectively. The biochemical evaluation showed that most pheochromocytomas had

1 hypersecretion of both metanephrine and normetanephrine (n=40, 58.8%), and radiologically, they  
2 generally presented an indeterminate mass with a median tumor size of 4.9 cm (IQR 3.6-6.5).

3         There was no difference in inflammation-based scores between patients with pheochromocytoma  
4 with and without hypertension, T2DM, CVE, and between incidentally detected and symptomatic  
5 patients (**Table 2**). Furthermore, tumors with PASS  $\geq 4$  (potentially aggressive behaviour) and those with  
6 lower PASS ( $< 4$ ) had similar inflammation-based scores; no significant gender differences were  
7 observed (**Table 2**). The presence of germline mutations did not influence the scores (**Table 2**), even  
8 when the specific genes were considered individually (data not shown). In contrast, inflammation-based  
9 scores differed based on ethnicity, BMI, and biochemical phenotype (**Table 2**). In particular, higher NLR  
10 and lower LMR levels were observed in Caucasian patients (p=0.005 and p=0.001, respectively)  
11 compared to subjects with other ethnicity; patients with obesity had higher NLR (p=0.02), PLR  
12 (p=0.0005) and SII (p=0.0009) and lower LMR (p=0.04) than those with BMI  $< 30$  kg/m<sup>2</sup> (**Table 2**).  
13 Catecholamine levels were further analysed in these two categories and significant differences in  
14 metanephrines were found between the ethnic groups. Caucasian patients had higher metanephrine levels  
15 than the other ethnic groups [2645 (906-5264) vs. 366.5 (173.3-1799), p=0.02], but the differences in  
16 NLR and LMR remained significant even after adjustment for plasma metanephrine levels and BMI  
17 (p=0.02 and p=0.002, respectively – data not shown). Although not significant, patients with lower BMI  
18 have higher catecholamine levels than obese patients [metanephrine 2155 (363-5200) vs 1073 (342.8-  
19 2225), p=0.2; normetaphrine 8201 (2683-25000) vs 5189 (1893-16986), p=0.6]. Considering the  
20 biochemical phenotype, we found that pheochromocytomas secreting only normetanephrines had lower  
21 NLR values (p=0.03) and higher LMR (p=0.03) and PNI (p=0.01) than those secreting both  
22 metanephrines and normetanephrines (**Table 2**).

23

24

25

## 1 *Hematological parameters in patients with pheochromocytoma, NFAT and EAH*

2 Patients with pheochromocytoma had a higher prevalence of pro-inflammatory changes  
3 compared to the NFAT and EAH groups, whilst no differences were found between NFAT and EAH  
4 (**Figure 1**). Patients with pheochromocytoma had higher leukocyte levels than EAH (median 7.4 [5.9-  
5 9.2] vs 6.3 [5.3-7.3];  $p=0.01$ ), mainly due to an increased neutrophil count (median 4.6 [3.6-5.6] vs 3.5  
6 [2.9-4.3];  $p=0.0002$ ). Neutrophil counts were also higher than in NFAT (4 [3.2-4.9];  $p=0.04$ ). In addition,  
7 patients with pheochromocytoma had a higher platelet count (median 275 [227.3-378.5]) than those with  
8 NFAT (255 [208.5-289],  $p=0.04$ ) and EAH (247 [208.5-291.5];  $p=0.01$ ). No significant differences were  
9 found in the lymphocyte, monocyte, and eosinophil counts.

10 Focusing on inflammation-based scores, NLR and PLR were higher in patients with  
11 pheochromocytoma (median 2.4 [1.8-3.9] and 165.8 [124.3-227.5], respectively) and decreased in  
12 patients with NFAT (median NLR 1.9 [1.6-2.3],  $p=0.009$ , and median PLR 135 [105.8-165.6],  $p=0.005$ )  
13 and EAH (median NLR 1.7 [1.2-2.3],  $p=0.0001$ , and median PLR 117.7 [94.2-150.2],  $p=0.0002$ ). The  
14 same trend was observed for SII (pheochromocytoma median 758.8 [416.1-1133] vs NFAT median  
15 489.3 [396.4-630],  $p=0.0006$  vs. EAH median 385 [312.7-564],  $p<0.0001$ ). Moreover, LMR values  
16 were lower in patients with pheochromocytoma (3 [2.3-3.8]) than in those with NFAT and EAH (3.9  
17 [3.3-4.6],  $p=0.0003$  and 3.9 [2.9-4.8],  $p=0.002$ , respectively) (**Figure 1B**).

## 18 19 *Relationship between hematological parameters and metanephrine levels and with CRP*

20 Plasma metanephrine levels significantly correlated with all the assessed inflammation-based  
21 scores (**Figure 2**). In particular, NLR ( $r= +0.463$ ,  $p=0.0002$ ), PLR ( $r= +0.317$ ,  $p=0.01$ ) and SII ( $r=$   
22  $+0.371$ ,  $p=0.004$ ) correlated positively, whereas LMR ( $r= -0.437$ ,  $p=0.0005$ ) and PNI ( $r= -0.374$   
23  $p=0.004$ ) correlated negatively. Apart from PLR, where the association did not reach statistical  
24 significance ( $p=0.054$ ), the correlations were confirmed after adjustment for age, sex, ethnicity, BMI and

1 tumor size of pheochromocytoma (**Supplementary Table 3**) (22). In contrast, normetanephrine levels  
2 did not correlate with inflammation-based scores, whilst a significant positive correlation with tumor size  
3 was found ( $r= +0.643$ ,  $p<0.0001$ , **Supplementary Figure 2**) (22).

4 Similarly, CRP was not associated with any of the inflammation-related scores evaluated (NLR  
5  $p=0.82$ ; PLR  $p=0.71$ ; LMR  $p=0.94$ ; SII  $p=0.74$ ; PNI  $p=0.25$ ).

6

### 7 *Haematological parameters in patients with pheochromocytoma after adrenalectomy*

8 Inflammatory parameters showed a significant improvement after more than one month of  
9 surgery and the subsequent resolution of catecholamine excess (median time 15.6 [5.7-42.3] months).  
10 Indeed, the lymphocyte count increased compared to baseline ( $p=0.01$ ), with corresponding changes in  
11 the relative inflammation-based scores (**Figure 3B**). Specifically, the postoperative NLR was  
12 significantly lower than the preoperatively (median values from 2.78 to 2.30,  $p=0.001$ ). The median PLR  
13 and SII also decreased from 201.2 to 152.7 ( $p=0.003$ ) and from 870.2 to 687.3 ( $p=0.004$ ), respectively.  
14 In addition, LMR increased from diagnosis (median values from 2.79 to 3.39,  $p=0.0002$ ). No difference  
15 was detected for other parameters of CBC (i.e., leukocyte, platelet, neutrophil, monocyte, eosinophil  
16 counts) and PNI ( $p=0.14$ , **Figure 3A**).

17 Looking at individual time points, the reduction in systemic inflammation showed a gradual  
18 progression after a transient increase immediately after surgery (median 3 [2-5] days). In detail, an  
19 increase in neutrophil and monocyte counts and a decrease in lymphocyte counts and serum albumin  
20 levels were observed at the first post-operative assessment (**Supplementary Figure 3A**) (22). This led to  
21 an increase in NLR ( $p<0.0001$ ) and SII ( $p=0.01$ ) and a decrease in MRL ( $p<0.0001$ ) and PNI  
22 ( $p<0.0001$ ) compared to the baseline (**Supplementary Figure 3B**) (22). Instead, as shown in  
23 **Supplementary Figure 4** (22), there was a significant reduction in NLR, PLR, and SII ( $p=0.0005$ ,  $0.003$ ,  
24 and  $0.006$ , respectively) during the first year after adrenalectomy (median time 5 months, IQR 1.8-7.9),  
25 which tended to decrease further at long-term follow-up (median time 39.6 months, IQR 26.4-55.2). In

1 addition, the LMR showed a consistent increase over time ( $p=0.03$  and  $0.02$  at short-term and long-term  
2 follow-up, respectively), while a decrease in PNI was only observed after one-year post-surgery  
3 ( $p=0.047$ ).

4

#### 5 *Influence of $\alpha$ -blocker therapy on the hematological parameters*

6 A subcohort of 29 patients with pheochromocytoma was assessed before and after treatment with  
7 preoperative  $\alpha$ -blockade (**Figure 4**). All patients were treated with doxazosin for a median of 110 (IQR  
8 78.5-261.5) days at a median total daily dose of 4 (IQR 2-11) mg. The changes in hematological  
9 parameters observed after doxazosin treatment were similar to those found in patients evaluated at least  
10 one month after surgery (**Figure 4**). In fact, lymphocytes were increased ( $p=0.01$ ) and neutrophils  
11 decreased ( $p=0.03$ ), resulting in a significant reduction in the NLR, with median values decreasing from  
12 2.63 to 2 ( $p=0.007$ ). As a result, SII, which is dependent on NLR, was also significantly reduced  
13 ( $p=0.03$ ). Furthermore, PLR values tended to be reduced after the introduction of  $\alpha$ -blockade ( $p=0.08$ ),  
14 whereas LMR and PNI were not different.

#### 15 **Discussion**

16 Hereby, we provide the first comprehensive study of the relationship between inflammation-  
17 based scores, as surrogates for systemic inflammation, and metanephrine levels in patients with  
18 pheochromocytoma compared to patients with NFAT or EAH. We studied a cohort of well-characterised  
19 patients with pheochromocytoma and, after confirming the presence of a preoperative systemic  
20 inflammatory state, we demonstrated the presence of a significant correlation between baseline plasma  
21 metanephrine levels and the inflammation-based scores. Moreover, we showed a positive effect of either  
22 the removal of the pheochromocytoma or the administration of  $\alpha$ -blockers drugs on these scores.

23 Previous studies have shown that patients with pheochromocytoma are characterised by the  
24 presence of pro-inflammatory changes. A review of almost 100 patients with catecholamine-secreting

1 tumors showed that leucocytosis and neutrophilia were a relatively common finding (15). Furthermore,  
2 other studies have found that not only CBC parameters but also acute-phase proteins, such as elevated C-  
3 reactive protein, were higher in patients with pheochromocytoma than healthy subjects or patients with  
4 other types of hypertensive conditions (9,14,28,29).

5 Our study is the first one assessing in a cohort of patients with pheochromocytoma and with the  
6 inclusion of control groups the levels of the systemic inflammation in patients using inflammation-based  
7 scores, markers proven to reflect inflammatory state in several diseases (17–19,21). Indeed, the previous  
8 study by Van der Heijden and co-authors suggested that patients with pheochromocytoma had higher  
9 levels of NLR and monocyte/lymphocyte ratio as compared with EAH patients, but the study was  
10 performed on only 10 patients (9). On the other hand, Zhong et al. analysed a large series of 728 patients  
11 with catecholamine-secreting tumors, but without comparison with any control groups, and investigating  
12 only the prognostic role of inflammation-based scores (16). At variance with those previous studies, we  
13 performed a comprehensive analysis of 68 patients with pheochromocytoma by assessing several  
14 inflammation-based scores and including a comparison with two control groups, i.e. 53 patients with  
15 EAH and 68 patients with NFAT. The fact that, significant changes in several inflammatory parameters  
16 were observed when comparing patients with pheochromocytoma with these two control groups, is of  
17 utmost importance. Indeed, as increased catecholamines are a hallmark of pheochromocytoma, the  
18 presence of an enhanced inflammatory state and inflammation-based scores, particularly the NLR (30–  
19 32), observed in these patients suggests that these hormones may have a greater impact on the systemic  
20 inflammatory response than hypertension or the presence of a tumor mass (33–35). This idea is supported  
21 by the observation that in our cohort of pheochromocytoma hypertension, CVE events, and T2DM did  
22 not significantly affect the inflammation-based scores.

23 As far as the clinical characteristics possibly influencing the inflammation-based scores in  
24 pheochromocytoma is concerned, we observed relationships between these parameters and both ethnicity  
25 and body weight. Specifically, Caucasian patients had more inflammatory changes, reflected by higher

1 NLR and lower LMR, than other ethnicities, consistent with previous observations in healthy subjects  
2 (36,37). Therefore, ethnicity may influence the clinical application of the present findings in the  
3 management of patients with pheochromocytoma, but the sample sizes analysed are not large enough to  
4 confirm this and further larger studies are required.

5 Interestingly, contrary to the conventional notion that obesity is associated with chronic  
6 inflammation (38,39), in our study patients with BMI <30 kg/m<sup>2</sup> showed a more pronounced systemic  
7 inflammatory state, as reflected by higher NLR, PLR, SII and low LMR values, than patients with  
8 obesity. In support of this finding, we observed that patients with a lower BMI tended to have higher  
9 levels of catecholamine, which are known to increase metabolic rate and induce weight loss (4,5,40).  
10 Indeed, in our cohort of pheochromocytoma, patients with a BMI <30 kg/m<sup>2</sup> tended to have higher  
11 catecholamine levels than obese patients, although no significant difference was found probably due to  
12 the limited number of patients. Thus, it is not possible to exclude that the apparently surprising more  
13 pronounced inflammatory state in patients with BMI <30 kg/m<sup>2</sup> could be due, in fact, to their  
14 tendentially higher catecholamine secretion, which seems to be associate with the inflammation-based  
15 scores.

16 Our study, indeed, is the first to demonstrate that plasma metanephrine levels are significantly  
17 correlated with all inflammation-based scores evaluated. In fact, we noticed a direct relationship, with  
18 increasing plasma metanephrine levels corresponding to increased NLR, PLR and SII, with concomitant  
19 decreases in LMR and PNI. Therefore, this finding suggests that catecholamines play a direct role in the  
20 systemic inflammation in pheochromocytoma, which is further supported by the lack of association  
21 found between scores and CRP levels. In particular, metanephrines seem to be more involved than  
22 normetanephrine, as evidenced by the lower inflammatory state in patients with pheochromocytomas  
23 secreting only normetanephrines. However, the possible different relationship between metanephrine or  
24 normetanephrine and inflammation has been poorly investigated and data reported in the literature are  
25 discordant. Overall, it seems that the presence of comorbidities (i.e., T2DM, insulin-resistance,

1 periodontitis, and obstructive sleep apnea syndrome) may play a role in influencing the relationship  
2 between metanephrine and inflammation (41,42).

3 The post-operative changes observed in the present study further support this hypothesis. The  
4 increase in both CBC and inflammation-based scores in the immediate postoperative period may be  
5 attributed to an acute stress response induced by the surgical procedure (43). However, we detected a  
6 significant reduction in inflammation during the long-term post-surgical monitoring, as also shown by  
7 other authors (9,14,16). In our study, these changes were more pronounced in the inflammation-based  
8 scores than CBC. In fact, at least one month after surgery, our patients showed a significant decrease in  
9 the lymphocyte count – and consequently NLR, PLR, SII increased and LMR decreased – suggesting a  
10 prevalent impact of lymphocyte compared to monocytes, platelets and neutrophils in this scenario. In  
11 summary, the resolution of changes in inflammation-based scores during long-term follow-up suggests  
12 an improvement in the inflammatory status of patients, with a potential benefit for their prognosis. This  
13 finding is in keeping with previous data showing that the postoperative reduction of NLR in patients with  
14 pheochromocytoma was associated with overall survival (16) and that the inflammation may play a role  
15 in the cardiovascular risk of patients with pheochromocytoma (9,14,44).

16 Finally, we investigated whether treatment with  $\alpha$ -blockers could influence the preoperative  
17 inflammation-based scores. So far, studies *in vitro* have shown that stimulation of  $\alpha$ -adrenergic receptors  
18 promotes the production of pro-inflammatory cytokines (45), which is inhibited by  $\alpha$ -adrenoceptor  
19 antagonists (46–48). Our study is the first to evaluate the influence of  $\alpha$ -blockade on inflammation-based  
20 scores in patients with pheochromocytoma. In a subset of 29 patients, we observed a reduction in NLR  
21 and SII with  $\alpha$ -blockers treatment and assumed a favourable influence on the inflammatory state of  
22 patients. However, we acknowledge that larger studies are needed to confirm these findings.

23 We recognize that our study has some limitations. First, due to its retrospective design, we  
24 cannot definitively establish a causal relationship between plasma metanephrine levels and inflammation-  
25 based scores, despite the observed association. Secondly, although our study extended the analysis by



1 including several inflammation-based scores compared to previous studies, we did not evaluate more  
2 specific inflammatory markers, such as circulating cytokines, interleukins, or acute-phase proteins.  
3 Measurement of these markers would provide a more complete assessment of the systemic inflammatory  
4 state in patients with pheochromocytoma. Furthermore, we did not have data on plasma chromogranin A,  
5 which could be a useful marker to associate with metanephrine levels. However, the sensitivity and  
6 specificity of this test are highly variable between studies, so its clinical use in pheochromocytoma  
7 remains an open issue (49). Thirdly, in our analysis we used a single blood count to calculate  
8 inflammation-based scores. Although we excluded any possible conditions that might have interfered  
9 with the test at the time of sampling, it would have been useful to have multiple measurements available  
10 to average for greater accuracy. Finally, the difference in tumor mass size between pheochromocytomas  
11 and NFAT may have affected the results of comparing these two groups. In fact, we found that  
12 pheochromocytoma were significantly larger than NFAT, and it is known that the tumor  
13 microenvironment can influence systemic inflammation (34,35). However, on univariate and multivariate  
14 analysis, we observed that the association between metanephrine levels and most inflammation-based  
15 scores was maintained even after adjustment for tumour size.

16 In conclusion, the association between plasma metanephrine levels and preoperative systemic  
17 inflammatory status, reflected by high NLR, PLR and SII as well as low LMR and PNI, that resolves  
18 during long-term follow up, suggests that the pro-inflammatory changes in pheochromocytomas are likely  
19 related to excessive amount of secreted catecholamines. The impact of circulating catecholamines on the  
20 systemic inflammatory response may play a role in the cardio-metabolic comorbidities in patients with  
21 pheochromocytoma. Understanding the connection between catecholamine levels, inflammation, and  
22 comorbidities may optimize treatment approaches, potentially improving outcomes and quality of life for  
23 individuals with pheochromocytoma. Further research is needed to confirm the exact mechanism by which  
24 catecholamines influence the systemic inflammation.

25

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3 Multidisciplinary team for their support in the management of patients with adrenal masses.

4  
5 **Data Availability**

6 All the relevant data underlying this article are available in the article and in its online supplementary  
7 material (22). Additional data will be shared on reasonable request to the corresponding author.

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### 7 **Legend to the Figures**

8

9 **Figure 1 – Full blood count and inflammation-based scores of patients with pheochromocytoma**  
10 **(PHEO, n=68), non-functioning adrenal tumor (NFAT, n=68) and essential hypertension (EAH,**  
11 **n=53).**

12 Comparison of full blood count (A) and inflammation-based scores evaluated (B) between patients with  
13 pheochromocytoma and two control groups. Data are reported as median and interquartile range, the upper  
14 and the lower whiskers represent respectively the 90 and the 10 percentiles. Statistical analysis was  
15 performed by Kruskal-Wallis test followed by Dunn's post hoc test (\* = p value < 0.03; \*\* = p value <  
16 0.002; \*\*\* = p value < 0.0001).

17 Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR,  
18 Lymphocytes-to-Monocytes Ratio; SII, Systemic Immune-Inflammation Index; PNI, Prognostic Nutrition  
19 Index; n, number.  
20

21 **Figure 2 – Correlation between inflammation-based scores with metanephrine levels in patients**  
22 **with pheochromocytoma at the time of diagnosis (n=59).**

1 Correlation analysis was performed by Spearman's test.

2 Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR,  
3 Lymphocytes-to-Monocytes Ratio; SII, Systemic Immune-Inflammation Index; PNI, Prognostic Nutrition  
4 Index; MN, metanephrine; n, number.

5  
6 **Figure 3 – Postoperative changes in complete blood count and inflammation-based scores in**  
7 **patients with pheochromocytoma.**

8 Changes in complete blood count (A) and inflammation-based scores (B) at the time of tumour diagnosis  
9 (*baseline*) and at two different times after adrenalectomy: on the last day of hospitalisation (*post-surgery*,  
10 n=68), more than one month after surgery (*last follow-up*, n=26). Statistical analysis was performed by  
11 Wilcoxon signed-rank test. Data are reported as median and interquartile range.

12 Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR,  
13 Lymphocytes-to-Monocytes Ratio; SII, Systemic Immune-Inflammation Index; PNI, Prognostic Nutrition  
14 Index; n, number.

15  
16 **Figure 4 – Changes in inflammation-based scores in patients with pheochromocytoma treated with**  
17 **preoperative  $\alpha$ -blockade (n=29).**

18 The changes in inflammation-based scores observed after  $\alpha$ -blockade treatment were similar to those  
19 found in patients evaluated at least one month after surgery (*last FU after surgery*).

20 Data are reported as median and interquartile range, the upper and the lower whiskers represent  
21 respectively the 90 and the 10 percentiles. Statistical analysis was performed by Wilcoxon signed-rank test  
22 (\* = p value < 0.05, \*\* = p value < 0.01).

1 Abbreviations: NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR,  
 2 Lymphocytes-to-Monocytes Ratio; SII, Systemic Immune-Inflammation Index; PNI, Prognostic Nutrition  
 3 Index; n, number.

4

5 **Table 1. Characteristics of the 68 patients with pheochromocytoma included in the study.**

6

<b>Parameter</b>	<b>Value</b>
<b>Median age (IQR), years</b>	53 (41.3-69)
<b>Gender, F/M (%F)</b>	44/24 (64.7)
<b>Ethnicity, n (%)</b>	
Caucasian	46 (67.6)
Non-Caucasian	10 (14.7)
Unknown	12
<b>Median BMI (IQR), kg/m<sup>2</sup></b>	26.7 (24-31.2)
<b>Active smoker, n (%)</b>	8 (11.8)
Unknown	18
<b>Germline mutations<sup>o</sup>, n (%)</b>	
Negative	28 (66.7)
Positive	14 (33.3)
- <i>RET (MEN2A)</i>	6 (8.8)
- <i>NF1</i>	3 (4.4)
- <i>Others*</i>	3 (4.4)
- <i>VHL</i>	2 (2.9)
Unknown**	26
<b>Presentation, n (%)</b>	
Incidentally discovered	32 (47.1)
Symptoms of pheochromocytoma	31 (45.6)
Screened for genetic susceptibility	5 (7.3)
<b>Cardiovascular-metabolic comorbidities, n (%)</b>	
CVE	13 (19.1)
HTN	52 (76.5)
T2DM	17 (25)
BMI >30 kg/m <sup>2</sup>	21 (30.9)
<b>Catecholamine excess, n (%)</b>	
Both MN and NMN	40 (58.8)
NMN	19 (27.9)
MN	7 (10.3)
Non-secreting	2 (2.9)
<b>Tumour laterality, n (%)</b>	
Unilateral <sup>#</sup>	61 (89.7)
Bilateral <sup>##</sup>	7 (10.3)
<b>MIBG scintigraphy avidity, n (%)</b>	
High	51 (75)
Negative	4 (5.9)
Unknown	13
<b>Median diameter (IQR), cm</b>	4.9 (3.6-6.5)
<b>Median Hounsfield Unit (IQR), n=19</b>	32 (29-41)

<b>Type of surgery, n (%)</b>	
Unilateral	61 (89.7)
Bilateral synchronous	4 (5.9)
Bilateral metachronous	3 (4.4)
<b>Median PASS Score (IQR), n=61</b>	5 (3-7.5)
<b>Median GAPP Score (IQR), n=11</b>	5 (4-6)
<b>Post-operative complications<sup>†</sup>, n (%)</b>	14 (20.6)

1 Categorical variables are reported as N (%); continuous variables are reported as median (IQR).  
2 °Genetic test for germline mutations was undertaken using Next Generation Sequencing of coding regions in  
3 *FH, MAX, MEN1, RET, SDHAF2, SDHA, SDHB, SDHC, SDHD, TMEM127*, and *VHL*; *NF1* was tested only in  
4 the presence of clinical features of neurofibromatosis type 1. \*Mutation in *MAX* gene in two patients and in  
5 *TMEM127* gene in one patient. \*\*Data are not available because patients: (i) have not eligibility criteria to  
6 genetic testing; (ii) did not provide consent; (iii) genetic testing was still ongoing. #Right-sided in 35 (51.5%)  
7 patients; Left-sided in 29 (42.6%) patients. ##For bilateral tumours, the maximum diameter of the larger adrenal  
8 mass was considered. †Complications occurring during hospital admission after surgery were hospital-acquired  
9 pneumonia in 7 patients, surgical wound infection in 2 patients, cardiac arrhythmia in 2 patients, urinary tract  
10 infection, type 2 respiratory failure and post-operative bleeding in 1 patient each.  
11 Legend: BMI, body mass index; MEN2A, Multiple Endocrine Neoplasia Type 2A; NF1, neurofibromatosis  
12 type 1; VHL, Von Hippel Lindau; CVE, cardiovascular events; HTN, hypertension, T2DM, type 2 diabetes  
13 mellitus; MN, metanephrine; NMN, normetanephrine; MIBG, meta-iodobenzylguanidine; PASS,  
14 Pheochromocytoma of the Adrenal Gland Scaled Score; GAPP, Grading system for Adrenal  
15 Pheochromocytoma and Paraganglioma; F, female; M, male; n, number; IQR interquartile range.  
16  
17

1 **Table 2. Relationship between serum inflammation-based scores and demographic, clinical and pathological characteristics of patients**  
 2 **with pheochromocytoma.**

	<b>NLR</b>	<b>p-value</b>	<b>PLR</b>	<b>p-value</b>	<b>LMR</b>	<b>p-value</b>	<b>SII</b>	<b>p-value</b>	<b>PNI</b>	<b>p-value</b>
<b>Gender</b>										
Male (n=24)	2.5 (1.8-3.9)	0.85	170.9 (131.7-221.8)	0.58	2.9 (2.3-3.4)	0.51	741.6 (468.0-942.6)	0.93	52.3 (47.6-55.8)	0.17
Female (n=44)	2.3 (1.6-3.8)		164.6 (112.8-230.4)		3.1 (2.3-4.1)		806.1 (376.7-1188)		54.5 (50.0-57.5)	
<b>Ethnicity (n=56)</b>										
Caucasian (n=46)	2.7 (1.9-4.4)	<b>0.005</b>	165.8 (124.9-229.6)	0.23	2.8 (2.2-3.4)	<b>0.001</b>	787.5 (449.3-1145.0)	0.12	53.2 (49.8-57.1)	0.37
Non-Caucasian (n=10)	1.6 (1-2.5)		131.2 (83.3-221.3)		4.6 (3.3-5.9)		616.5 (275.2-945.1)		55.3 (50.4-58.4)	
<b>Symptoms of pheochromocytoma</b>										
Yes (n=31)	2.3 (1.7-3.3)	0.37	164.6 (112.4-222.0)	0.28	3.1 (2.5-3.8)	0.29	742.9 (375.0-1167.0)	0.57	56 (51.5-58.0)	0.05
No (n=37)	2.6 (1.8-4.2)		170.6 (133.3-247.5)		2.9 (2.2-3.6)		806.1 (515.3-958.4)		52.5 (47.8-55.0)	
<b>Incidental mass</b>										
Yes (n=32)	2.4 (1.8-4.2)	0.73	174.3 (131.8-260.9)	0.15	2.9 (2.1-3.4)	0.24	813.8 (511.2-1172.0)	0.40	51.5 (46.8-55.8)	0.06
No (n=36)	2.5 (1.7-3.6)		159.8 (112.6-217.7)		3.1 (2.5-3.8)		738.7 (376.0-1133.0)		54.8 (51.6-57.5)	
<b>Germline mutations</b>										
Negative (n=28)	2.2 (1.8-2.9)	0.16	159.6 (124.6-210.4)	0.97	2.8 (2.3-3.4)	0.46	656.1 (388.2-897.3)	0.33	53.5 (49.3-59.1)	0.26
Positive (n=14)	2.1 (1.9-4.6)		166.9 (99.5-207.3)		3.2 (2.2-4.3)		870.2 (407.9-1703)		55.5 (53.3-58.6)	
<b>CVE</b>										
Yes (n=13)	2.6 (1.8-4.8)	0.55	221.1 (149.9-258.6)	0.17	2.8 (2-3.8)	0.59	837.5 (583.8-1349.0)	0.48	51.5 (46.3-56)	0.12
No (n=55)	2.4 (1.8-3.3)		154.9 (118.0-222.0)		3 (2.4-3.8)		734.5 (399.1-973.6)		54 (50.0-57.5)	
<b>HTN</b>										
Yes (n=52)	2.7 (1.8-4.2)	0.11	176.7 (119.6-240.9)	0.15	3 (2.3-3.8)	0.85	821.4 (468.0-1342.0)	0.07	53 (49.3-57.5)	0.58
No (n=16)	2 (1.6-2.8)		138.7 (124.4-176.6)		2.9 (2.5-3.8)		543.3 (384.2-894.0)		54.5 (51.8-56.0)	
<b>T2DM</b>										
Yes (n=17)	2.8 (1.5-3.8)	0.95	169.1 (117.6-246.7)	0.72	3.2 (2.4-4.7)	0.31	845.7 (502.5-1399.0)	0.57	53.5 (51.0-56.9)	0.68
No (n=32)	2.3 (1.8-3.7)		176.7 (125.9-249.3)		3 (2.2-3.6)		716.1 (468.0-1154.0)		52.5 (47.9-57.9)	
<b>Obesity</b>										
BMI <30 (n=46)	2.6 (1.9-4.6)	<b>0.02</b>	186.1 (138.5-242.2)	<b>0.0005</b>	2.9 (2.2-3.8)	<b>0.04</b>	874.7 (562.9-1393.0)	<b>0.0009</b>	52.5 (48.0-57.0)	0.17
BMI ≥30 (n=19)	2 (1.4-2.9)		124.5 (82.8-173.9)		3.3 (2.7-4.7)		456 (329.3-833.5)		55 (50.8-58.3)	
<b>Catecholamine excess</b>										
Both MN-NMN (n=40)	136 (106.2-168)	*0.03	518.9 (493.5-548.8)	*0.41	130.4 (115.7-144.6)	*0.03	672.1 (634.5-720.5)	*0.43	396.6 (387.4-402.3)	*0.01
NMN (n=19)	109.9 (92.4-132.6)	#0.99	493.8 (449-540.7)	#0.99	148.2 (134.6-179.2)	#0.99	638.2 (589.3-701.8)	#0.99	406.9 (396.1-411.9)	#0.99
MN (n=7)	115.5 (97.3-172.9)	°0.99	497.7 (461.5-517.3)	°0.42	155.8 (116.3-207.9)	°0.22	643 (554.7-679.8)	°0.30	402.5 (398-406.9)	°0.64
<b>Median mass size</b>										
<4.9 cm (n=34)	2.4 (1.8-1.7)	0.65	166.4 (124.0-224.0)	0.99	2.8 (2.3-3.8)	0.52	742.9 (471.1-939.8)	0.64	52.5 (47.3-55.9)	0.28
≥4.9 cm (n=34)	2.6 (1.7-4.0)		160.1 (124.3-237.4)		3.2 (2.5-3.7)		787.5 (387.3-1355.0)		54.3 (50.4-57.0)	

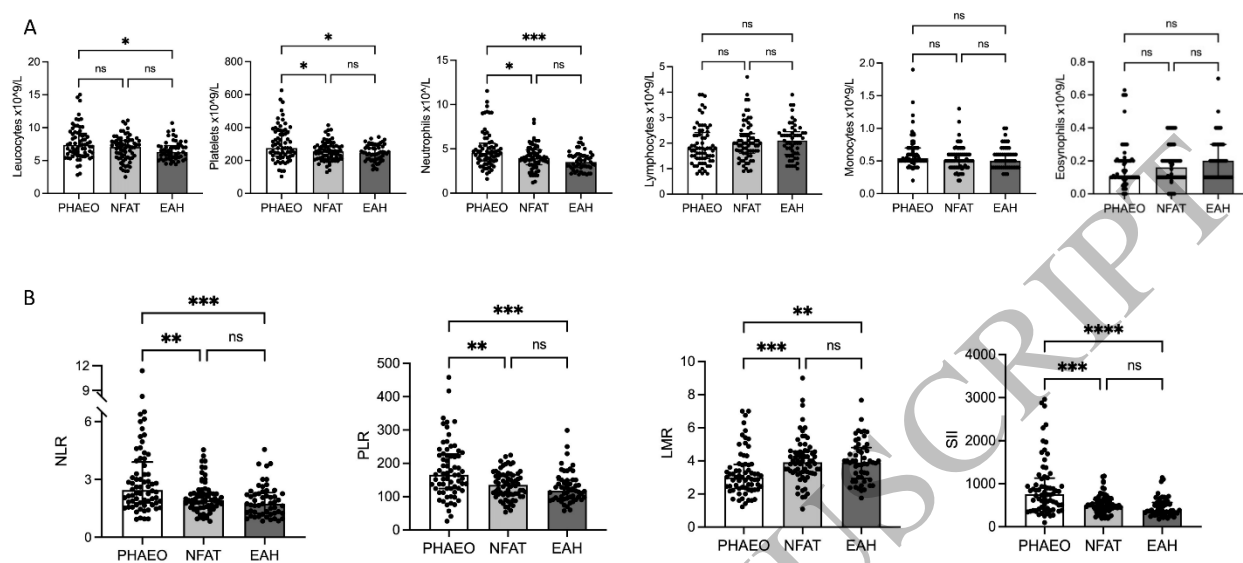
<b>PASS</b>										
PASS $\leq$ 4 (n=25)	2.3 (1.7-4.2)	0.77	136.7 (94.7-230.4)	0.18	3.2 (2.3-4.1)	0.41	562.9 (360.8-1153.0)	0.09	55 (50.8-58.0)	0.61
PASS $>$ 4 (n=36)	2.6 (1.7-4.1)		170.3 (138.0-221.8)		2.9 (2.3-3.6)		848.3 (586.5-1208.0)		53.3 (50.1-57.0)	

1 Continuous variables are reported as median (IQR) and statistical analysis were performed by two-tailed Mann-Whitney U test. \*NMN vs Both MN-  
2 NMN, #NMN vs MN, °MN vs Both MN-NMN.  
3 Legend:NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; LMR, Lymphocytes-to-Monocytes Ratio; SII, Systemic Immune-  
4 Inflammation Index; PNI, Prognostic Nutrition Index; phaeo, pheochromocytoma; CVE, cardiovascular events; HTN, hypertension, T2DM, type 2  
5 diabetes mellitus; BMI, body mass index; MN, metanephrine; NMN, normetanephrine; PASS, Pheochromocytoma of the Adrenal Gland Scaled Score; n,  
6 number.

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ACCEPTED MANUSCRIPT

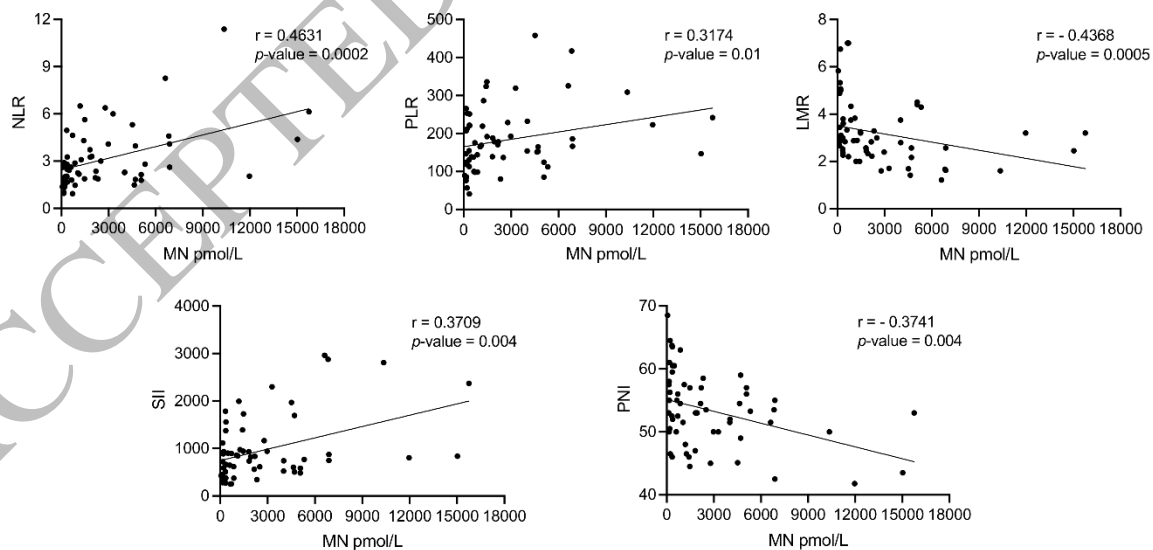
Figure 1



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Figure 1  
339x190 mm (DPI)

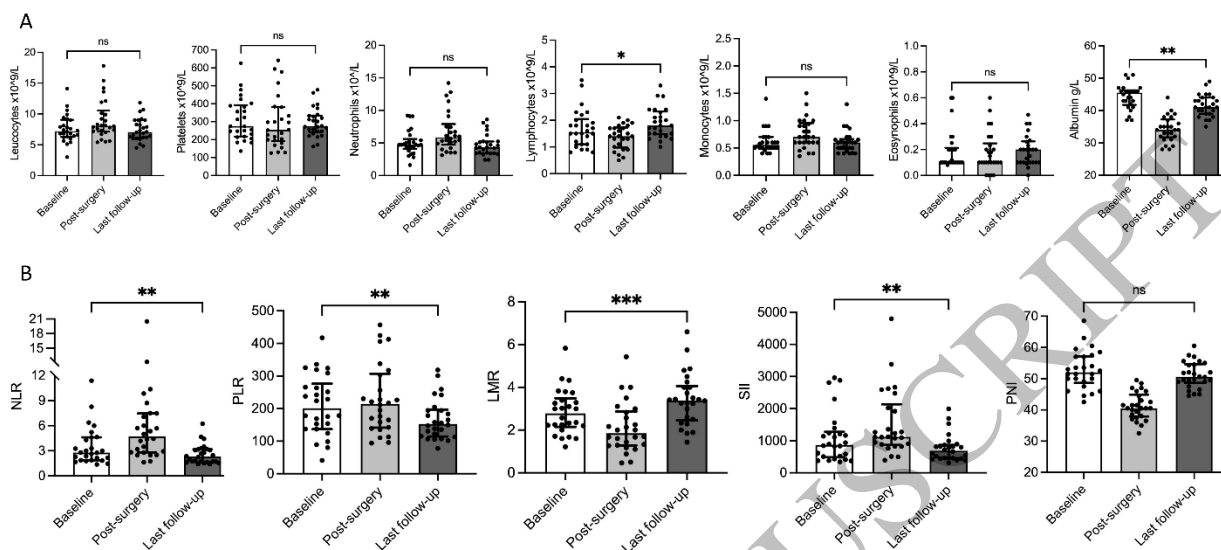
Figure 2



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Figure 2  
339x190 mm (DPI)

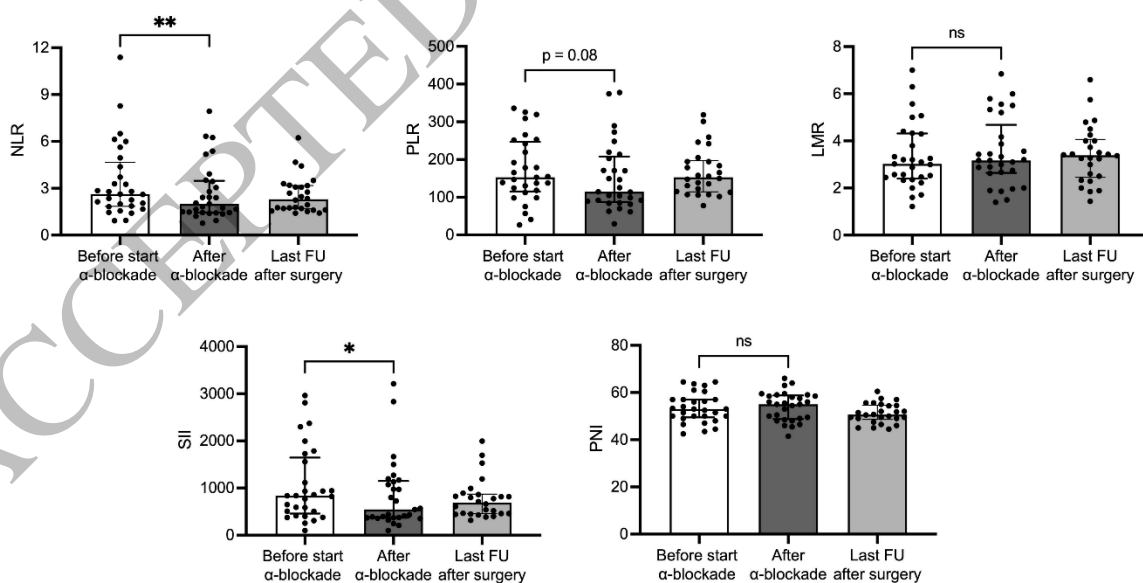
Figure 3



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Figure 3  
559x314 mm (DPI)

Figure 4



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Figure 4  
559x314 mm (DPI)