Journal of Clinical Medicine

MDPI

Article

Methylprednisolone induced lymphocytosis: a prospective study in 20 patients with immune mediated inflammatory disorders

Camille Beniada, Bruno Couturier [1], Florence Reye[2], Cédric Delporte [2], Pierre Van Antwerpen [2] , Viviane De Maertelaer [3] and Elie Cogan [1*]

[1] *Department of Internal Medicine, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium;*

[2] *RD3-Pharmacognosy, bioanalysis and drug discovery and Analytical Platform of the Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium.*

[3] *Service de Biostatistique et Informatique Médicale, Université libre de Bruxelles, Brussels, Belgium*

[*] *Correspondence:* elie.cogan@ulb.be, Tel : 00 32 475412737

Abstract: A morning lymphocytosis has been observed in patients under methylprednisolone (mPDN) treatment. We here determine prospectively the timing and magnitude of mPDN-induced lymphocytosis and study the effects of concomitant beta-blockers administration on lymphocyte count (L). L was measured before and 24 to 72 hours after initiating mPDN treatment in 20 patients with immune-mediated inflammatory disorders (IMID). After one week, patients with increased L were divided in two groups receiving, in addition to mPDN, either propranolol or a placebo; L was determined 4 days later. Lymphocyte subpopulations and mPDN plasma levels were determined in subsets of the patients. Values are expressed as median with 25%-75% interquartile range. A 73.4 % (37-305) increase of L was observed in 18/20 patients as soon as 48 (48-72) hours after initiating mPDN (32 mg; 16-32). Lymphocytosis (L ≥4,000/µL) was observed in 7 patients and hyperlymphocytosis (L ≥ 5,000/µL) in 4 of them. No significant changes in L variation were shown under propranolol. In conclusion, the morning lymphocytosis observed during mPDN treatment occurs in the very first days of mPDN administration. The lack of effect of propranolol does not support the role of an increased adrenergic tone.

Keywords: Glucocorticoids, lymphocytosis, methylprednisolone, beta-blockers, adrenergic tone, IMID

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *J. Clin. Med.* **2022**, *11*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

1. Introduction

Glucocorticoids are anti-inflammatory and immunosuppressive agents that play a key role in the treatment of most IMID. Irrespective of the underlying disease, they induce metabolic effects such as hyperglycemia and hypokalemia, as well as hematological alterations [1]. Glucocorticoids induce a transient neutrophilia mainly by a decrease of neutrophils adhesion to endothelial cells, a decrease of eosinophil and basophil count and an inhibition of the action and differentiation of macrophages [2]. Transient lymphopenia occurring a few hours after glucocorticoid intake is a well-known and studied phenomenon [3].

Our group previously reported that chronic mPDN administration to patients suffering from IMID was associated to a morning lymphocytosis, identified, in this retrospective study, thirteen days after starting the treatment [4]. All T-cell subpopulations were involved, mainly CD4 cells [4]. Due to the retrospective nature of

this study, the precise timing of lymphocytosis remained uncertain, and the underlying mechanism could not be determined. Among the proposed hypotheses, the increased adrenergic tone induced by glucorticoids could contribute to this mPDN-induced morning lymphocytosis [4].

The link between corticosteroids and beta-adrenergic receptors is a well-known phenomenon; it has been reported, especially in asthmatic patients for whom oral prednisolone treatment produces an up-regulation of beta-adrenergic receptors on lymphocytes [5].

Methylprednisolone-induced lymphocytosis might therefore result from an increased adrenergic tone combined with a "transient morning glucocorticoid insufficiency", 24 hours after oral mPDN intake. Indeed, at that time, residual concentrations of mPDN are very low and endogenous secretion of cortisol is still inhibited [4].

Our study focuses on patients with IMID in whom a treatment with mPDN is introduced. The purpose of the present study was to investigate prospectively in a subset of patients with IMID the time course of white cell movements under mPDN treatment and to determine if concomitant beta-blocker administration can influence mPDN-induced lymphocytosis.

2. Materials and Methods

2.1. Patients

Twenty patients (8 men and 12 women aged 24-94 years), with a diagnosis of IMID, requiring a treatment with methylprednisolone (Médrol®, Pfizer) were included.

Exclusion criteria were as follows: contraindication for beta-blockers (asthma, severe chronic obstructive pulmonary disease, second- and third-degree atrioventricular block), treatment that could interfere with beta-blockers (verapamil, diltiazem, ion exchanging resin), concomitant immunosuppressive therapy, age < 18 years, lymphocyte count <1000/mm^3 at the beginning of treatment and intake of beta-blockers before mPDN therapy.

2.2. Methylprednisolone

The dose of methylprednisolone was generally ≤ 0.6 mg/kg body weight. The inclusion of patients with an initial higher dosage could be delayed until the dosage reached the threshold of 0.6 mg/kg body weight.

2.3. Beta-blocker

Propranolol, a non-selective ß1-ß2 beta-blocker (40 mg two times a day) was chosen. Heart rate was recorded in the same conditions (at resting, same time of the day, sitting) before and after initiation of propranolol.

2.4. Blood tests

First, before mPDN administration, lymphocyte count (L) was determined in the morning and, in a subset of the patients, in the afternoon, 7-8 hours later.

Under treatment, L was determined in the morning, before taking mPDN, i.e., 24 hours after taking the last previous mPDN dose and in the afternoon, about 7-8 hours after mPDN intake

2.5. Timing and groups

The study was organized as follows:

- A three-day pre-treatment control period
- a first period (from day 1 to day 5) at the beginning of mPDN therapy.

- a second period (from day 6 to day 12), during which the patients with an increased L were randomly assigned to two groups: group 1, patients treated with mPDN receiving propranolol (Inderal®, Astra Zeneca) 40 mg two times a day and group 2, patients treated with mPDN receiving the placebo. Five days after propranolol or placebo administration, L was determined in the morning before mPDN administration.

2.6. Definitions

The pre-treatment lymphocyte count (L_0) was an average of 2 measurements performed before the beginning of the mPDN treatment.

L_{AM1} and L_{AM2} are the morning lymphocyte count (cells/µL) respectively in period 1 and period 2.

We defined « lymphocytosis » as L ≥ 4,000/µL and « hyperlymphocytosis» as L ≥ 5,000/µL according to the reference [4].

2.7. Blood Analysis

Each blood sample was treated at the laboratory within two hours.

Differential blood count was determined by the routine laboratory.

CD3, CD4, CD8, CD19 and natural killer cells were obtained by flow cytometry (Navios, Beckman Coulter [Brea, Calif], 3 lasers, 10 colors) in Erasme's laboratory of clinical hematology.

mPDN concentrations were measured as described previously [4]. Before and after mPDN intake, the glucocorticoid was measured by liquid chromatography-tandem mass spectrometry with a triple quadripole 6490 equipped with a AJS-ESI source (Agilent technologies, Palo alto, CA, USA) at the Analytical Platform of the Faculty of Pharmacy, ULB.

2.8. Statistical Analysis

Considering the number of patients included in the present study and the lack of normal distribution of the data (according to the Kolmogorov-Smirnov and Shapiro-Wilk tests), non-parametric tests were chosen for statistical analysis: Wilcoxon matched-pairs signed rank test and Mann Whitney U test for unpaired data. The results were considered significant at $P < .05$.

Data are expressed as the median (25% - 75% interquartile range) unless otherwise specified.

3. Results

3.1. Study population

The present study included 8 men and 12 women (median age 53 years; range, 24-94 years), recently diagnosed with an IMID: vasculitis (including giant cell arteritis, Behcet's disease, aortitis; n=6), sarcoidosis (n=4), adult Still's disease (n=2), dermatological affections with inflammatory component (n=3) , inflammatory lung diseases (n=2), IgG4 disease (n=1), Devic's disease (n=1), serum sickness (n=1).

The median initial daily dose of mPDN was 32 mg (16-32). 2 of 4 patients initially treated with higher dose of mPDN (> 0.6 mg/kg) were followed and included when mPDN dose was reduced. Demographic and clinical characteristics of the study population can be found in Supplementary Table S1.

3.2. Effects of mPDN on lymphocyte count

For the whole group (table 1), a significant 1.78-fold rise in morning lymphocyte count was observed after 48 hours (range: 24-72) of treatment (n= 20; $p < 0.001$). As no

increase of the lymphocyte count was observed in patients #3 and #13, 18 patients out of 20 (90%) developed an increase of the lymphocyte count [LAM1 3143 (2487-4298) vs L0 1681 (1477-2167); p<0.001].

Table 1: Effect of mPDN on morning lymphocyte count

Patient	Initial dose	dose/kg	Dose L_{AM1}	t (hours)	L_0	L_{AM1}	ΔL_{AM1}
# 1	32	0.43	32	48	2108	3289	1181
# 2	16	0.21	16	72	3247	4130	883
# 3	32	0.38	32	48	2320	2300	-20
# 4	48	0.64	32	48	2537	6890	4353
# 5	16	0.32	16	72	1275	2200	925
# 6	24	0.38	24	24	1070	1470	400
# 7	48	0.69	32	48	1607	5130	3523
# 8	32	0.53	32	72	1539	2580	1041
# 9	32	0.40	32	48	2342	3654	1312
# 10	32	0.53	32	48	1688	3066	1378
# 11	48	0.74	48	24	2417	5452	3035
# 12	16	0.23	16	72	1596	3048	1452
# 13	12	0.17	12	48	1805	1682	-123
# 14	32	0.43	32	48	1673	3220	1547
# 15	32	0.58	32	72	1491	1999	508
# 16	16	0.19	16	48	1898	3311	1413
# 17	8	0.10	8	48	1738	2208	470
# 18	32	0.53	32	72	1433	2808	1375
# 19	48	0.69	48	24	1185	4800	3615
# 20	16	0.23	16	48	1881	2583	508
Min	8	0.10	8	24	1070	1470	-123
Max	48	0.74	48	72	3247	6890	3053
Median	32	0.41	32	48	1713	3057	1247
P25	16	0.23	16	48	1503	2231	508
P75	32	0.57	32	72	2267	4011	1523
P Value						$P < 0.001$	

Initial dose: dose of mPDN (mg)

Dose L_{AM1}: mPDN dose is equivalent of the initial dose except for patient #4 and #7; in those patients the initial mPDN was higher than 0.6 mg/kg and increased lymphocytosis was delayed occurring after a reduction of mPDN dose below 0.6 mg/kg

t: duration of mPDN administration at the time of the blood test (except for patients #4 and #7) (See above)

L_{AM1} : Morning lymphocyte count 24 to 72 hours after beginning of mPDN treatment (cells/µL)

ΔL_{AM1}: Variation in lymphocyte count (cells/µL)

Min: minimum value

Max: maximum value

P25: 25th percentile

P75: 75th percentile

During the first 3 days of mPDN administration, lymphocytosis ($L_{AM1} \geq 4{,}000$ cells/µL) was observed in 5 patients and hyperlymphocytosis ($L_{AM1} \geq 5{,}000$ cells/µL) in 3 of them.

In addition, during the second period, two more patients developed a lymphocytosis ($L_{AM2} \geq 4{,}000$ cells/µL) and one patient with lymphocytosis during period 1 developed hyperlymphocytosis during period 2. Thus, the lymphocyte count was ≥ 4,000/µL in 7 patients out of 20 (35%) with hyperlymphocytosis in 4 of them.

Of the 4 subjects included with a 48 mg initial mPDN dose (thus > 0.6 mg/kg), two (patients #4 and #7) did not show an increase in their lymphocyte count during the first 72 hours. The lymphocytosis appears respectively 48 hours after decreasing mPDN dosing to 32 mg (table 1).

As mentioned above, 2 patients (patients #3 and # 13) who began their treatment with mPDN oral intake <0.6 mg/kg, did not develop an increase of their lymphocyte count during this period. Therefore, they were not included for the second part of the study.

In addition to the morning blood test, L was also measured in the afternoon in 8 subjects during both the control period and the first 3 days of mPDN administration (figure 1). The increase in morning L contrasts with the well-known lymphopenic effect of mPDN documented by the blood test performed 7-8 hours after mPDN administration.

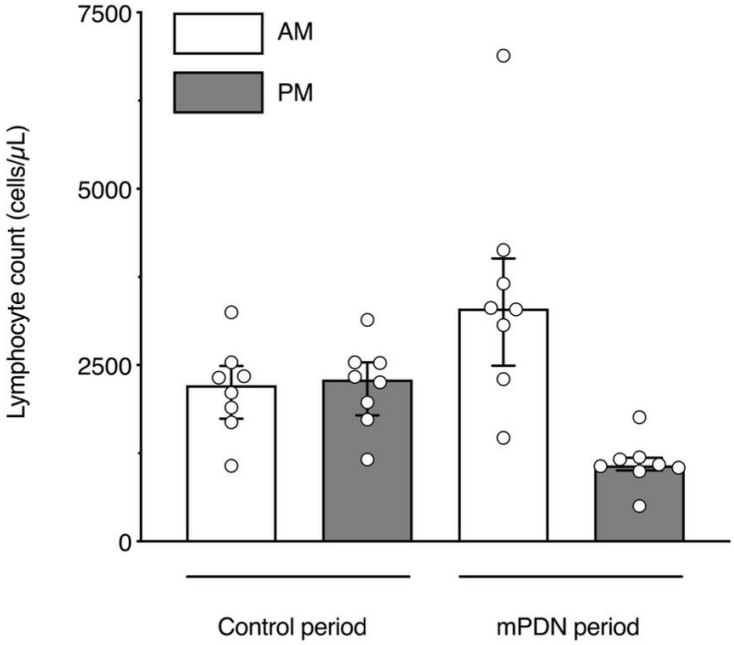


Figure 1. Effect of methylprednisolone on lymphocyte count

Columns correspond to the median values and error bars to the 25-75% interquartile range.

White columns represent morning lymphocyte counts et gray columns values of the lymphocyte counts in the afternoon, 7-8 hours after the morning blood test.

The first group of columns are the values before the administration of mPDN; the second group of columns represent the values 24 to 72 hours after the beginning of mPDN treatment.

Open circles are individual data available in a subgroup of 8 subjects.

3.3. Effect of mPDN on morning lymphocyte sub-populations

Lymphocyte immunophenotyping was determined in 10 patients, before and after the initiation of mPDN treatment to study the changes in lymphocyte subpopulations. The increase in morning L involved all lymphocyte subpopulations except NK cells. For T cells, the absolute increase in CD4 cells was significantly more important than for CD8 cells (689 cells/µL vs 168 cells/µL; p= 0.002). However, the CD4/CD8 ratio before treatment (CD4/CD8: 2.51) was not statistically different after mPDN administration (CD4/CD8: 2.64) (table 2).

Table 2. Effect of mPDN on morning lymphocyte subpopulations

	Total Ly		CD3		CD4		CD8		CD19		NK	
Patient	L_{AM0}	**L_{AM1}**	L_{AM0}	**L_{AM1}**	L_{AM0}	**L_{AM1}**	L_{AM0}	**L_{AM1}**	L_{AM0}	**L_{AM1}**	L_{AM0}	**L_{AM1}**
# 1	2108	3289	1253	2100	940	1615	258	420	313	580	14	290
# 2	3247	4130	2169	3457	1669	2847	472	581	411	520	688	500
# 4	2537	6890	1696	5469	1153	3621	452	1850	249	1404	316	517
# 6	1070	1470	582	792	437	556	128	193	94	380	19	237
# 7	1607	5130	1025	3931	634	1595	379	1164	100	818	454	517
# 9	2342	3654	1391	2350	920	1623	409	620	389	1071	266	48
# 16	1898	3311	1455	1904	1195	1479	221	371	217	895	298	505
# 17	1738	2208	1481	1529	879	899	582	595	80	353	172	298
# 19	1185	4800	948	3709	649	2593	258	964	208	341	49	454
# 20	1881	2583	1423	2092	908	1394	446	619	205	335	69	129
Median	1890	3483	1407	2225	914	1605	394	607	213	550	219	376
P value	0.002		0.002		0.002		0.002		0.002		0.109	

The effect of mPDN on lymphocyte subpopulations was obtained in 10 out of the 20 patients of the study.
Patient identification is noted in the first column.
Total Ly: total lymphocytes
L_{AM0}: morning cell counts before treatment; L_{AM1}: morning cell counts, 24-72 hours after the beginning of mPDN treatment.
Values are expressed in absolute cells number per µL.
Statistical significance obtained by Wilcoxon matched pairs signed rank test.

3.4. Effect of mPDN administration on morning monocytosis

Along with lymphocytosis, a significant increase in monocyte count was observed (P =0.007). After 24 to 72 hours, 80% of the subjects developed a rise in the monocyte count above pre-treatment values. Five subjects developed a moderate monocytosis (M_{AM1} > 700 cells/µL) and 4 subjects a frank monocytosis (M_{AM1} >1000 cells/µL).

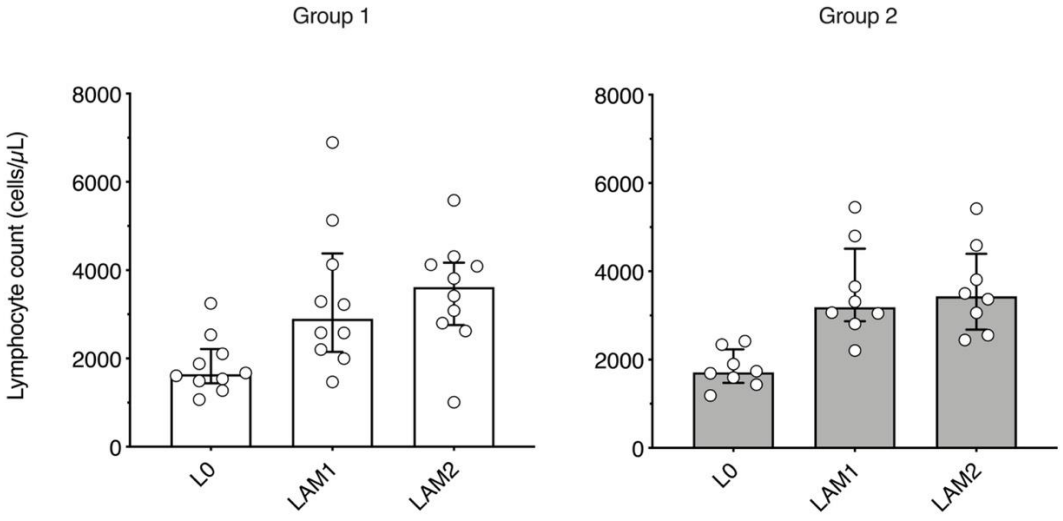
3.5. Effect of propranolol on the lymphocyte count

This second part of the study concerned 18 subjects (10 for group 1 and 8 for group 2).

All patients from group 1 showed a significant decrease of heart rate (bpm, beats per minute) after five days of treatment with propranolol, ensuring propranolol efficacy (67 bpm vs 80 bpm; p=0.002)

In group 1, administration of propranolol did not affect morning lymphocytosis. No significant difference in morning lymphocyte count (L_{AM}) was observed between the two periods (L_{AM1} = 2902 cells/µL vs L_{AM2} = 3614 cells/µL; P = 0.490).

No difference in lymphocyte count variation was shown between group 1 and group 2 (P = 0.570) (figure 2).



214

Figure 2. Effect of propranolol on lymphocyte count 215

Columns correspond to the median values and error bars the 25-75% interquartile range. 216

L0 represents the lymphocyte count before mPDN administration. 217

LAM1 represents the lymphocyte count during the 3 first days of mPDN administration 218

LAM2 represents the lymphocyte count during the second period of the treatment: group 1, patients on propranolol (n=10); group 2, patients on placebo (n=8) 219 220

No significant difference between LAM1 and LAM2, neither between group 1 nor group 2 221

3.6. Methylprednisolone and cortisol plasma levels 222

Methylprednisolone and cortisol plasma levels could be obtained for 15 patients out of 20. The median residual morning concentration of mPDN was as low as 2.44 ng/mL as compared to 88 ng/ml 8 hours after mPDN administration. 223 224 225

The median morning cortisol level was also low (60.1 ng/mL; normal range: 100-250 ng/mL). 226 227

4. Discussion 228

If transient acute reversible lymphopenia secondary to glucocorticoid administration is a well-known phenomenon that has been described on several occasions [1-3], the morning lymphocytosis associated with mPDN administration in patients with IMID is a poorly known phenomenon that was previouly reported in a retrospective study [4]. 229 230 231 232

In the present prospective study of 20 patients, we show that mPDN administration induced a significant increase in morning lymphocyte count, above pre-treatment values in 90% of the patients. 7 patients out of 20 even developed a lymphocytosis (cells ≥ 4,000/µL) and hyperlymphocytosis (cells ≥ 5,000/µL) in 4 of them. The absence of mPDN induced lymphocytosis in 2 out of 20 patients could be explained by a selective mPDN resistance in one patient [6] and a possible lack of compliance in the other. 233 234 235 236 237 238

In the previous retrospective study of Bromberg et al, the lymphocytosis associated with mPDN treatment could only be noticed 13 days after the beginning of the treatment corresponding to the first available blood test after the introduction of mPDN [4]. 239 240 241

The prospective nature of the present study allows us to demonstrate that the phenomenon occurs as soon as 24 to 72 hours after initiation of the treatment. Moreover, previous observations suggested that morning lymphocytosis does not appear with a mPDN dosing above 0.6 mg/kg. In the present study, the increase in the morning lymphocyte count was delayed in two patients with a higher initial mPDN dose and occurred when the mPDN dose was reduced. We hypothesize that the residual morning 242 243 244 245 246 247

plasma level of mPDN could be still sufficiently high to impede the lymphocytosis phenomenon to occur. The same reason might be evoked to explain that a lymphocytosis was not observed in one of the two non-responding patients.

Thus, in most of the patients, morning lymphocytosis developing in the first 3 days of mPDN treatment provides a guarantee for treatment observance. In contrast, the absence of morning increase in lymphocyte count might suggest therapeutic non-compliance or drug interaction interfering with mPDN catabolism.

Such a timing has already been suggested in healthy subjects treated with prednisolone during 3 consecutive days and who had undergone a blood test 24 hours after mPDN intake, a crucial time to notice this phenomenon [7]. Along the same lines, Ten Berge et al. show, in healthy subjects, a rebound lymphocytosis developing 24 hours after administration of 10 to 60 mg of prednisolone [8].

4.1. Influence of mPDN administration on morning lymphocyte subpopulations

Significant increase in T lymphocyte count, especially CD4 cells, had previously been reported by Bromberg et al [4]. By contrast, in that study, rise in B lymphocyte count did not reach the significant threshold, an increase in CD19 cells count was present in only 6 patients out of 8. The present study demonstrates that morning lymphocytosis involves both B and T cells.

In addition, our study shows that morning monocyte count also rises significantly under mPDN treatment. Morning monocytosis following glucocorticoid administration had been clearly, but not systematically, reported in the literature. Shoenfeld et al. illustrated, in patients with long-term prednisone treatment, a rebound monocytosis occurring 24 hours after drug intake [9]. A significant monocytosis was also noticed 24 hours after the administration of oral prednisone in healthy subjects [10].

4.2. Hypothetical mechanisms

The possibility that the morning lymphocytosis observed after methylpredsinolone treatment may be related to modulation of adrenergic tone is suggested by a range of evidence.

The link between glucocorticoids and adrenergic tone explains some of the side effects of glucocorticoid treatment (tremor, tachycardia, etc.) and is supported by studies on the effects of glucocorticoids on adrenoceptors. Davies and Lefkowitz have demonstrated an increase of beta-adrenergic receptors on lymphocytes 24 hours after intake of 100 mg of oral hydrocortisone, at the time where residual concentrations are very low [11].

An increased adrenergic tone also explains the lymphocytosis occurring after exercise and, in healthy subjects, propranolol administration for one week reduces the lymphocytosis observed after a physical activity [12].

For athletes, the increase in lymphocyte count observed after intense physical exercise is the result of catecholamine release and is accompanied by an increase of beta-adrenergic receptors density on the surface of lymphocytes [13]. In 2009, Dimitrov et al. demonstrated, in healthy subjects, the specific and contrasting effect of cortisol and adrenaline on T cells circadian changes [14]. They suggest a negative correlation between the absolute count of naïve T-cells and circulating cortisol; in contrast, physiological catecholamine release contribute to an increase of circulating CD8 lymphocytes.

By analogy with athlete's lymphocytosis, we hypothesized that excessive adrenergic stimulation combined with an increase of beta-adrenergic receptor on lymphocytes induced by mPDN could induce an increase in circulating lymphocytes.

To test this hypothesis, we selected propranolol as a possible inhibitor of the morning mPDN induced lymphocytosis. Propranolol, a non-selective ß1-ß2 beta-blocker was chosen, according to its use in recent studies dealing with lymphocytosis resulting from

physical activity or mental stress [12,15]. It is suggested that lymphocytosis resulting from physical activity depends on ß2-adrenergic mechanisms. Indeed, it could be reduced in subjects treated with 40 mg propranolol whereas this was not the case in subjects who received metoprolol, a ß1 betablocker.

Our results do not confirm that hypothesis. The lack of lymphocytosis reduction with propranolol administration suggests another pathophysiological mechanism responsible for mPDN induced lymphocytosis.

The mechanisms responsible for lymphocytes circulation from the blood to lymphoid tissues are largely unknown. A study undertaken on guinea pigs with radioactive chromium (Cr51) labelled lymphocytes showed a significant movement of T and B lymphocytes to the bone marrow, leading to lymphopenia [16]. Furthermore, in healthy subjects, it has been demonstrated that infusion of physiological dose of epinephrine leads to a decrease in the adhesion molecule CX3CR1 on CD8 lymphocytes, potentially explaining their redistribution to the circulation [14].

It therefore appears reasonable to assume that the increase in lymphocyte count 24 hours after drug intake is also due to a redistribution of lymphocytes to the circulation, coming from different body compartments including the bone marrow and possibly lymph nodes and spleen.

The lack of effect of propranolol administration invalidates the hypothesis of a main role of adrenergic tone in these lymphocytic shifts.

The alternative hypothesis is that relative matinal glucocorticoid insufficiency could lead to a rebound lymphocytosis. Cortisol has already been described has a “regulator of the number of lymphocytes in peripheral blood”, with a negative correlation between absolute lymphocyte count and cortisol level [14,17]. What is more, patients with adrenal insufficiency whose cortisol levels are very low, may present an associated lymphocytosis [18] which may concern up to 50% of the patients in Addison’s disease [19]. By analogy with adrenal insufficiency, in the study of Bromberg and al., lymphocytosis appears at the time when residual levels in mPDN were very low and endogenous cortisol almost suppressed [4]. These results were confirmed in the present study. Indeed, the residual median morning concentration of mPDN was only 2.8 % as compared to mPDN concentration a few hours after mPDN administration. Morning cortisol plasma levels were also largely below the normal range according to adrenal inhibitory effect of exogenous glucocorticoid administration.

By contrast, high residual morning concentration (resulting from slow metabolism or high mPDN dosing) could explain the absence of lymphocytosis in patients treated with mPDN dosage exceeding 0.6 mg/kg body weight as shown in some patients in the present study. That hypothesis is supported by the observation of Fauci et al who have studied the effects of several glucocorticoids on lymphocyte and monocyte counts in healthy humans: morning lymphocytosis does not appear after a single high dose of 80 mg of prednisone [10].

5. Conclusions

The results of this prospective study confirm a significant rise in morning lymphocyte count in 90% of the patients with IMID and treated with mPDN, 7 of them (near 40%) developing a lymphocytosis (≥ 4,000 cells/µL) or an hyperlymphocytosis (≥ 5,000 cells/µL)

This phenomenon appears in the first one to three days of treatment and involves both T and B lymphocytes. The development of lymphocytosis or hyperlymphocytosis in these conditions should therefore not prompt clinicians to perform invasive and unnecessary investigations.

On the contrary, the absence of morning lymphocytosis in the setting of mPDN treatment is unusual. It could occur with high methylprednisolone dosage or must question on the compliance to the treatment or possible interaction with mPDN metabolism.

The pathophysiological mechanisms remain undetermined so far. The lack of effect of propranolol administration does not support a possible role of the adrenergic pathway.

Supplementary materials

The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: title

Author contributions: Conceptualization, Camille Beniada, Bruno Couturier and Elie Cogan; Formal analysis, Florence Reye; Funding acquisition, Cedric Delporte; Investigation, Camille Beniada, Florence Reye, Cedric Delporte and Pierre Van Antwerpen; Methodology, Camille Beniada, Bruno Couturier and Elie Cogan; Resources, Cedric Delporte; Software, Viviane De Maertelaer; Supervision, Elie Cogan; Validation, Camille Beniada; Writing – original draft, Camille Beniada; Writing – review & editing, Camille Beniada and Elie Cogan.

All authors have read and agreed to the published version of the manuscript

Funding: This research was founded by the Belgian National Fund for Scientific Research (FRS, N° 3.4553.08 and T.0136.13 PDR) and the Université libre de Bruxelles (FER-2007)

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Erasme Hospital's institutional review board (Erasme reference: P2017/454)

Informed Consent Statement: A written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable

Conflicts of Interest: The authors declare no conflict of interest

References

1. Claman, H.N. Corticosteroids and lymphoid cells. *N Engl J Med* **1972**, *287*, 388-397, doi:10.1056/NEJM197208242870806.
2. Boumpas, D.T.; Chrousos, G.P.; Wilder, R.L.; Cupps, T.R.; Balow, J.E. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. *Ann Intern Med* **1993**, *119*, 1198-1208, doi:10.7326/0003-4819-119-12-199312150-00007.
3. Yu, D.T.; Clements, P.J.; Paulus, H.E.; Peter, J.B.; Levy, J.; Barnett, E.V. Human lymphocyte subpopulations. Effect of corticosteroids. *J Clin Invest* **1974**, *53*, 565-571, doi:10.1172/JCI107591.
4. Bromberg, L.; Roufosse, F.; Pradier, O.; Delporte, C.; Van Antwerpen, P.; De Maertelaer, V.; Cogan, E. Methylprednisolone-Induced Lymphocytosis in Patients with Immune-Mediated Inflammatory Disorders. *Am J Med* **2016**, *129*, 746-752 e743, doi:10.1016/j.amjmed.2016.02.013.
5. Tan, K.S.; McFarlane, L.C.; Lipworth, B.J. Effects of oral and inhaled corticosteroid on lymphocyte beta2-adrenoceptor function in asthmatic patients. *Br J Clin Pharmacol* **1997**, *44*, 565-568, doi:10.1046/j.1365-2125.1997.00627.x.
6. Drigo, I.; Piscianz, E.; Valencic, E.; De Iudicibus, S.; Tommasini, A.; Ventura, A.; Decorti, G. Selective resistance to different glucocorticoids in severe autoimmune disorders. *Clin Immunol* **2010**, *134*, 313-319, doi:10.1016/j.clim.2009.11.005.
7. Pountain, G.D.; Keogan, M.T.; Hazleman, B.L.; Brown, D.L. Effects of single dose compared with three days' prednisolone treatment of healthy volunteers: contrasting effects on circulating lymphocyte subsets. *J Clin Pathol* **1993**, *46*, 1089-1092, doi:10.1136/jcp.46.12.1089.
8. ten Berge, R.J.; Sauerwein, H.P.; Yong, S.L.; Schellekens, P.T. Administration of prednisolone in vivo affects the ratio of OKT4/OKT8 and the LDH-isoenzyme pattern of human T lymphocytes. *Clin Immunol Immunopathol* **1984**, *30*, 91-103, doi:10.1016/0090-1229(84)90010-2.
9. Shoenfeld, Y.; Gurewich, Y.; Gallant, L.A.; Pinkhas, J. Prednisone-induced leukocytosis. Influence of dosage, method and duration of administration on the degree of leukocytosis. *Am J Med* **1981**, *71*, 773-778, doi:10.1016/0002-9343(81)90363-6.
10. Fauci, A.S. Mechanisms of corticosteroid action on lymphocyte subpopulations. II. Differential effects of in vivo hydrocortisone, prednisone and dexamethasone on in vitro expression of lymphocyte function. *Clin Exp Immunol* **1976**, *24*, 54-62.
11. Davies, A.O.; Lefkowitz, R.J. Corticosteroid-induced differential regulation of beta-adrenergic receptors in circulating human polymorphonuclear leukocytes and mononuclear leukocytes. *J Clin Endocrinol Metab* **1980**, *51*, 599-605, doi:10.1210/jcem-51-3-599.
12. Murray, D.R.; Irwin, M.; Rearden, C.A.; Ziegler, M.; Motulsky, H.; Maisel, A.S. Sympathetic and immune interactions during dynamic exercise. Mediation via a beta 2-adrenergic-dependent mechanism. *Circulation* **1992**, *86*, 203-213, doi:10.1161/01.cir.86.1.203.
13. Maisel, A.S.; Harris, T.; Rearden, C.A.; Michel, M.C. Beta-adrenergic receptors in lymphocyte subsets after exercise. Alterations in normal individuals and patients with congestive heart failure. *Circulation* **1990**, *82*, 2003-2010, doi:10.1161/01.cir.82.6.2003.

14. Dimitrov, S.; Benedict, C.; Heutling, D.; Westermann, J.; Born, J.; Lange, T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood* **2009**, *113*, 5134-5143, doi:10.1182/blood-2008-11-190769.
15. Benschop, R.J.; Nieuwenhuis, E.E.; Tromp, E.A.; Godaert, G.L.; Ballieux, R.E.; van Doornen, L.J. Effects of beta-adrenergic blockade on immunologic and cardiovascular changes induced by mental stress. *Circulation* **1994**, *89*, 762-769, doi:10.1161/01.cir.89.2.762.
16. Fauci, A.S. Mechanisms of corticosteroid action on lymphocyte subpopulations. I. Redistribution of circulating T and b lymphocytes to the bone marrow. *Immunology* **1975**, *28*, 669-680.
17. Thomson, S.P.; McMahon, L.J.; Nugent, C.A. Endogenous cortisol: a regulator of the number of lymphocytes in peripheral blood. *Clin Immunol Immunopathol* **1980**, *17*, 506-514, doi:10.1016/0090-1229(80)90146-4.
18. De La Balze, F.A.; Reifenstein, E.C., Jr.; Albright, F. Differential blood counts in certain adrenal cortical disorders (Cushing's syndrome, Addison's disease and panhypopituitarism). *J Clin Endocrinol Metab* **1946**, *6*, 312-319, doi:10.1210/jcem-6-4-312.
19. Baez-Villasenor, J.; Rath, C.E.; Finch, C.A. The blood picture in Addison's disease. *Blood* **1948**, *3*, 769-773.