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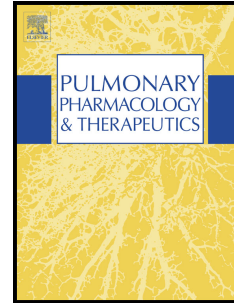
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Effects of glucocorticoids on the neutrophil count: a cohort study among hospitalized patients

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Abstract

Background: Systemic glucocorticoids are often used in clinical practice for a large variety of indications. Clinical observations have shown that patients using glucocorticoids often have higher neutrophil counts. Debate remains whether this observed neutrophilia is associated with glucocorticoid use or that other factors, like disease and severity of disease, should be considered. The objective of this study was to investigate the effect of systemic glucocorticoids on the absolute neutrophil count in hospitalized patients.

Methods: A cohort study was conducted using data from the Utrecht Patient Oriented Database which comprises clinical data of patients of the University Medical Center Utrecht. We identified all adult patients, hospitalized in 2005 with at least two blood samples for hematological testing during admission and compared in hospital glucocorticoid use with non-use.

Results: A total of 809 glucocorticoid users and 2,658 non-users were included in the study with comparable neutrophil counts at admission ($8.2 \cdot 10^9/l$ for glucocorticoid users and $8.0 \cdot 10^9/l$ for non-users). Overall analysis showed a slight association between glucocorticoid use and an increase in neutrophil count (RR 1.3; 95% CI 1.1-1.5). However, within diagnostic subgroups there was no increase in neutrophil count in glucocorticoid users. Furthermore, among all no dose response relationship, no effect of time between the two samples, and no effect of anti-inflammatory/sodium retaining potency was found.

Conclusion: Observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than glucocorticoid use itself.

Key words: cohort studies; glucocorticoid; immunology; neutrophil

Abbreviations: DTA, difficult-to-treat asthma; GC, glucocorticoid; UPOD, Utrecht Patient Oriented Database

1. Introduction

Glucocorticoids (GCs) have a widespread and complex mechanism of action. They have many effects, depending on disease and cell type [1, 2]. Glucocorticoids reduce the number of many inflammatory cells, in particular increase the rate of apoptosis of eosinophils [1-5].

Neutrophils were found to be less responsive to these drugs [6-8]. In vitro studies have shown that glucocorticoids inhibit neutrophil apoptosis [1, 2, 9-11]. However, in vivo studies mainly have focused on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count and showed normalization of the neutrophil count within 24 hours [12, 13]. Therefore, the clinical effect of glucocorticoids on the neutrophil count is controversial.

Clinical observations have shown that patients using GCs often have higher neutrophil counts, particularly in respiratory disease. It is well known that granulocytes play an important role in asthma and COPD [14, 15] but although neutrophilia has been associated with asthma severity, debate remains whether neutrophilia is a characteristic of asthma severity or results from glucocorticoid treatment [5, 8, 16-24]. More severely ill asthma patients receive often higher doses of glucocorticoids and glucocorticoids are highly used among hospitalized asthma patients. Therefore, in this observational study, we compared neutrophil counts in a variety of diseases in patients using GCs with non-users, taking into account several approaches to adjust for disease bias on these counts.

2. Material and methods

2.1 Study design and setting

A cohort study was conducted using data from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising administrative data on patient characteristics, laboratory test results, medication orders, discharge diagnoses and

medical procedures for all patients treated at the University Medical Center Utrecht, a 1,042 bed tertiary teaching hospital in the center of the Netherlands. Each year, approximately 165 000 patients are treated during more than 28 000 hospitalizations, 15 000 day care treatments, and 334 000 outpatient visits. UPOD data acquisition and data management is in accordance with current Dutch privacy and ethical regulations. A more complete description of UPOD has been published elsewhere [25].

We identified all adult patients (≥ 18 years) who were hospitalized in the University Medical Center Utrecht in 2005 and had at least two hematological blood tests during hospitalization. Exposed patients used systemic glucocorticoids during admission, where glucocorticoid use started before or after withdrawal of the first blood test; unexposed patients did not use systemic glucocorticoids during admission. The study design is summarized in Figure 1. For all participants the discharge diagnose was defined according to the ICD-9-CM code [26].

2.2 *Neutrophil testing*

For each glucocorticoid user, the first blood test during admission and the last blood measurement during in-hospital glucocorticoid use were selected for analysis, where these samples should cover at least a one day period. Up to four unexposed patients were sampled to each glucocorticoid user according to calendar time (with a maximum of 15 days before or after the test date of the user), neutrophil count at time of admission (max $2 \cdot 10^9$ neutrophils/l around the neutrophil count of the user) and days between the two blood samples (max two days around the number of days for the glucocorticoid user). We used data on eosinophil counts in the same patients measured on the same moments in time as a reference procedure as the effect of glucocorticoids on the absolute eosinophil count is widely known [1-5]. Data on glucocorticoid use was retrieved from the medication file from UPOD and daily dose

exposure was expressed as systemic prednisone equivalents, using defined daily dosages [27]. Moreover, glucocorticoids were categorized according to their anti-inflammatory/sodium retaining potency for subgroup analyses [28].

2.3 Data analysis

Student t-tests, Mann-Whitney tests, and chi-square tests were used, as appropriate.

Glucocorticoid dose and change in neutrophil and eosinophil counts were associated through a non-linear relationship. Therefore, the glucocorticoid dose was categorized into tertiles. The change in neutrophil and eosinophil count was also categorized into tertiles to obtain three equally sized groups. These three groups were defined as an increase, decrease or no change in neutrophil or eosinophil count. Potential confounders included in the analysis were age, gender, diagnosis, duration of hospitalization, length of period between two blood samples, CRP levels, and death during hospitalization.

Subsequently, unconditional multivariate Cox proportional hazards analysis was used to estimate the strength of the association between glucocorticoid exposure and either an increase versus no change, or a decrease compared to no change in the absolute neutrophil or eosinophil count, expressed as relative risk (RR) with 95% confidence intervals (CI). All variables that changed the regression coefficient of glucocorticoid use with less than ten percent were excluded from the model. All analyses were conducted using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

From the UPOD database, we identified 809 glucocorticoid users and 2,658 non-users. The age and gender distributions of the glucocorticoid users were comparable to patients not using

systemic glucocorticoids, as well as the duration of hospitalization and the number of deaths during hospitalization. CRP levels were comparable for GC users and non-users at time of admission, but CRP levels were lower for GC users in the second blood sample compared to non-users (Table 1). Glucocorticoid users had a lower eosinophil count than non-users, and non-users had an increase in eosinophil count during hospitalization (Table 1, Figure 2). The absolute neutrophil count at admission was comparable for users and non-users. During hospitalization, glucocorticoid users had an overall minor increase of $0.3 \cdot 10^9$ neutrophils/l, where non-users had an overall slight decrease of $0.3 \cdot 10^9$ /l (Table 1). However, differences in neutrophil count between the two blood samples for each patient were equally distributed among glucocorticoid users and non-users, with means close to zero (Figure 2). As shown in Table 1, glucocorticoid use was not randomly distributed among the diagnosis groups.

As shown in Table 2, 28.1% of users and 32.5% of non-users had a decrease of more than $2 \cdot 10^9$ neutrophils/l, 40.7% of users and 42.4% of non-users remained unchanged and 31.3% of users and 25.1% of non-users had an increase of more than $2 \cdot 10^9$ neutrophils/l. Overall, use of systemic glucocorticoids was associated with a slight increase in the neutrophil count (crude RR 1.2; 95% CI 1.0-1.4). After adjustment for diagnosis, the adjusted RR yielded a value of 1.3 (95% CI 1.1-1.5). However, subgroup analysis for the major diagnostic groups showed that there is no association between glucocorticoid use and an increase in neutrophil count among diagnostic subgroups. The RR for neoplasms was 1.2 (95% CI 0.8-1.6), for circulatory disease 1.2 (95% CI 0.8-1.6), and for respiratory diseases 1.1 (95% CI 0.6-2.1).

Glucocorticoid use was not associated with a decrease in the absolute neutrophil count in overall analysis or in subgroup analyses (Table 2). With respect to the absolute eosinophil count 220 (27.3%) of users and 534 (20.1%) of non-users had a decrease of more than $0.05 \cdot 10^9$ /l, 366 (45.4%) of users and 893 (33.6%) of non-users remained unchanged and 220

(27.3%) of glucocorticoid users and 1,227 (46.2%) of non-users had an increase of more than $0.05 \cdot 10^9/l$. Use of systemic glucocorticoids was inversely associated with an increase in the eosinophil count (adjusted RR 0.6; 95% CI 0.6-0.7). There was no clear association of glucocorticoid use and a decrease in eosinophil count (adjusted RR 1.2, 95% CI 1.0-1.4).

The number of days between the two blood samples for each patient varied between one and 77 days between user-non-users pairs. As shown in Figure 3, there was no effect of the time between the blood samples for each patient and the change in neutrophil count, nor were there any differences for glucocorticoid users and non-users. Regarding short-term effects, there was a wide variation in the change in neutrophil counts for glucocorticoid users as well as for glucocorticoid non-users with a one day period between the two blood samples. A sensitivity analysis revealed that limiting the maximum period between the two blood samples to seven days did not influence the results (data not shown).

Furthermore, different diagnoses require treatment with different doses of glucocorticoids, which could cause confounding in studying the relationship between glucocorticoids and the neutrophil count. Among glucocorticoid users, no dose response relationship could be found, where adjustment occurred for diagnosis (Table 3). Of the glucocorticoid users 474 (58.6%) used glucocorticoids at time of the first blood sample, 335 (41.4%) of the users started glucocorticoid treatment after withdrawal of the first blood sample. Stratification on this parameter did not influence the overall results with an RR 1.9 (95% CI 1.4-2.5) for an increase versus no change compared with 1.3 (95% CI 1.1-1.5) when including all patients. For a decrease versus no change, the RRs were 0.7 (95% CI 0.5-1.0) for only users that started glucocorticoid use after the first blood sample compared with 0.9 (95% CI 0.8-1.1) when all patients were included in the model. Subgroup analyses for the major diagnostic groups

among patients that started glucocorticoid use after the first blood sample showed no association between glucocorticoid use and an increase or decrease in neutrophil count (data not shown). Stratifying glucocorticoids according to their anti-inflammatory/sodium retaining potency did not yield different results (data not shown).

4. Discussion

In this study, we showed that observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than with the use of glucocorticoids itself. Overall analysis yielded a 30% increase in the risk of an increase in the neutrophil count for GC users (adjusted RR 1.3, 95% CI 1.1-1.5). However this is the summed effect of several factors, next to the possible effect of systemic glucocorticoids, e.g. diagnosis, disease severity, dose and type of glucocorticoid, and the studied time window. These factors are considered one by one.

Regarding diagnosis, subgroup analysis in the major diagnostic groups showed that there was no association between glucocorticoid use and a change in the absolute neutrophil count among diagnostic subgroups.

Concerning disease severity, glucocorticoid users have a higher absolute neutrophil count in the second blood sample, compared to non-users, and this count is above the upper limit of our laboratories normal reference range of $1.6 - 8.3 \cdot 10^9$ neutrophil/l, possibly indicating that glucocorticoid users were more severely ill than non-users (Table 1). However, the mean change in absolute neutrophil count for GC users, as well as for non-users was close to zero (Figure 2) and demographic parameters and disease severity markers, like duration of hospitalization and death during hospitalization, were comparable between GC users and non-

users, CRP levels were lower in the second blood sample for GC users compared to non-users (Table 1).

Another argument showing that neutrophilia is not fully due to glucocorticoid use is that effect of dosing and anti-inflammatory potency of the glucocorticoid was not found in our study. Also, a sensitivity analysis showed that varying the cut-off value of a change in neutrophil count from 1 to $10 \cdot 10^9/l$ did not have major effects on the associations found (data not shown). The inverse association found between glucocorticoid use and an increase in eosinophil count is reassuring as it served as an internal reference procedure of our database.

The time window of studying the effect of glucocorticoids on the neutrophil count is important. Early, non-genomic effects occur within minutes to seconds after administration, genomic mechanisms of action take 30 minutes to 18 hours [1, 29, 30]. In vivo studies mainly focused on healthy volunteers and short-term effects of glucocorticoids on the neutrophil count within 24 hours [12, 13]. Because of the clinical relevance of a prolonged change in the neutrophil count in this study there is at least a one day period between the two blood samples of each patient. The second blood sample for users was selected during glucocorticoid use.

Our findings are in accordance with other studies that found that neutrophilia is not fully due to glucocorticoid treatment. Green et al. concluded that, in some asthma subjects at least, neutrophilia is not due to the glucocorticoid treatment [31]. Also Louis et al. found in their study that severe asthmatics, treated with systemic glucocorticoids, had a lower absolute neutrophil count in sputum compared to severe asthmatics who did not use systemic glucocorticoids [18]. Further research is needed to study inflammation in order to create more insight into the mechanistic role of neutrophils in asthma severity.

One might argue that the results of this study should be verified in a randomized controlled trial. However, such a trial will probably not be approved by any medical ethical committee, because severe asthma patients cannot be deprived of glucocorticoids since these drugs are essential in the treatment of asthma and there is no alternative treatment at this time.

There are several studies on the cellular mechanism of glucocorticoids in general [2, 6, 32-35]. In spite of the discussions about the glucocorticoid effect on neutrophils, this mechanism is not well-understood and should be studied in more detail. There are several mechanisms by which glucocorticoids could theoretically influence the absolute neutrophil count. By increasing the neutrophil production in the bone marrow, by demargination from the blood vessel wall, by increasing the life span, by limiting neutrophil emigration from the blood, or by a combination of these factors [1, 9, 10, 12, 28]. The bone marrow could be activated to produce more neutrophils [12, 28]. However, when the bone marrow is producing granulocytes more quickly, it will also release immature granulocytes into the blood, which is not the case in our study population. Factors like stress, exercise, or infection could cause demargination of neutrophils close to the vessel wall, resulting in an increase in the neutrophil count in peripheral blood, but glucocorticoids are not likely to cause demargination [12, 28, 36]. Glucocorticoids were found to inhibit neutrophil apoptosis [1, 2, 9-11]. However, these findings were done in in-vitro experiments, and in-vivo studies only found short-term effects of glucocorticoids on the neutrophils [12, 13]. Lastly, glucocorticoids could reduce the membrane CD11/CD18 appearance, causing weakened adhesion and less neutrophils leaving the blood [12, 37].

In conclusion, the overall increased risk of an increase in neutrophil count in this study is the summed effect of several factors. The observed neutrophilia in users of systemic glucocorticoids is probably associated with underlying disease, rather than the use of glucocorticoids itself.

ACCEPTED MANUSCRIPT

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Table 1. Baseline characteristics of glucocorticoid users and non-users*

Characteristic	GC users (n=809)	Non-users (n=2,658)	P-value (two-sided)
Age, yr	57.4 ± 17.8	58.4 ± 18.3	0.171 ^a
Sex			0.092 ^c
Male	411 (50.8)	1,440 (54.2)	
Female	398 (49.2)	1,218 (45.8)	
Days between blood samples	6 (3-12)	4 (2-8)	<0.001 ^b
Duration of hospitalization	10 (7-19)	10 (7-18)	0.332 ^b
Death during hospitalization	54 (6.8)	157 (5.9)	0.360 ^c
Absolute eosinophil count first blood sample (10 ⁹ /l)	0.07 (0.03-0.17)	0.09 (0.04-0.18)	< 0.001 ^b
Absolute eosinophil count second blood sample (10 ⁹ /l)	0.07 (0.03-0.15)	0.15 (0.06-0.27)	< 0.001 ^b
Absolute neutrophil count first blood sample (10 ⁹ /l)	8.2 ± 4.7	8.0 ± 4.2	0.214 ^a
Absolute neutrophil count second blood sample (10 ⁹ /l)	8.5 ± 5.1	7.7 ± 4.1	<0.001 ^a
CRP first blood sample measured	311 (38.4)	1,196 (45.0)	
CRP (mg/l)	29.0 (7.0-75.0)	21.0 (7.0-68.8)	0.094 ^b
CRP second blood sample measured	236 (29.2)	285 (10.7)	
CRP (mg/l)	23.5 (9.0-62.0)	33.0 (13.0-99.0)	0.001 ^b
Diagnosis			<0.001 ^c
Neoplasms	251 (31.0)	261 (9.8)	
Diseases of the circulatory system	93 (11.5)	1,023 (38.5)	
Diseases of the respiratory system	109 (13.5)	108 (4.1)	
Infectious and parasitic diseases	30 (3.7)	63 (2.4)	
Endocrine, nutritional and metabolic diseases, and immunity disorders	22 (2.7)	53 (2.0)	
Diseases of the nervous system and sense organs	30 (3.7)	65 (2.4)	
Diseases of the digestive system	44 (5.4)	198 (7.4)	
Diseases of the genitourinary system	73 (9.0)	95 (3.6)	
Diseases of the skin, subcutaneous tissue, musculoskeletal system, and connective tissue	36 (4.4)	148 (5.6)	
Other	121 (14.9)	644 (24.2)	

*Data are presented as n (%), or mean ± SD, or median (interquartile range)

^a Student t-test; ^b Mann-Whitney test; ^c χ^2 test

Table 2. Association between glucocorticoid use and a change in absolute neutrophil count

	GC users N (%)	Non-users N (%)	Crude RR (95% CI)	Adjusted RR (95% CI) ^a
Overall analysis N=809 N=2,658				
No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	329 (40.7)	1127 (42.4)	Reference	Reference
Increase ($\geq 2.10^9/l$)	253 (31.3)	667 (25.1)	1.2 (1.0-1.4)	1.3 (1.1-1.5)
Decrease ($\leq -2.10^9/l$)	227 (28.1)	864 (32.5)	0.9 (0.8-1.1)	0.9 (0.8-1.1)
Neoplasms N=251 (31.0) N=261 (9.8)				
No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	114 (45.4)	119 (45.6)	Reference	
Increase ($\geq 2.10^9/l$)	86 (34.3)	71 (27.2)	1.2 (0.8-1.6)	
Decrease ($\leq -2.10^9/l$)	51 (27.2)	71 (27.2)	0.8 (0.6-1.2)	
Circulatory disease N=93 (11.5) N=1,023 (38.5)				
No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	29 (31.2)	399 (39.0)	Reference	
Increase ($\geq 2.10^9/l$)	38 (40.9)	376 (36.8)	1.2 (0.8-1.6)	
Decrease ($\leq -2.10^9/l$)	26 (28.0)	248 (24.2)	1.2 (0.8-1.8)	
Respiratory disease N=109 (13.5) N=108 (4.1)				
No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	47 (43.1)	37 (34.3)	Reference	
Increase ($\geq 2.10^9/l$)	22 (20.2)	15 (13.9)	1.1 (0.6-2.1)	
Decrease ($\leq -2.10^9/l$)	40 (36.7)	56 (51.9)	0.8 (0.5-1.1)	

GC = glucocorticoid

^a Adjusted for diagnosis (in case of overall analysis)

Table 3. Dose effect among glucocorticoid users, stratified according to the dose used (in mg prednisone equivalents) before the first blood sample and the mean daily dose used between the two blood samples

GC use before the first blood sample	Mean daily dose of GC use between the two blood samples	Increase ($\geq 2.10^9/l$)	No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	Crude RR (95% CI)	Adjusted RR (95% CI) ^a
0	< 15	40 (30.3)	51 (38.9)		
	15 – 40	44 (33.3)	35 (26.7)	1.3 (0.8-1.9)	1.3 (0.8-2.0)
	≥ 40	48 (36.4)	45 (34.4)	1.2 (0.8-1.8)	1.1 (0.7-1.8)
0 - 50	< 15	19 (51.4)	49 (56.3)		
	15 – 40	13 (35.1)	27 (31.0)	1.2 (0.6-2.4)	1.8 (0.8-4.1)
	≥ 40	5 (13.5)	11 (12.6)	1.1 (0.4-3.0)	1.6 (0.5-4.9)
≥ 50	< 15	1 (1.2)	7 (6.3)		
	15 – 40	27 (32.1)	29 (26.1)	3.9 (0.5-28.4)	4.5 (0.6-34.2)
	≥ 40	56 (66.7)	75 (67.6)	3.4 (0.5-24.7)	3.6 (0.5-26.4)
GC use before the first blood sample	Mean daily dose of GC use between the two blood samples	Decrease ($\leq -2.10^9/l$)	No change ($-2.10^9/l < \text{change} < 2.10^9/l$)	Crude OR (95% CI)	Adjusted OR (95% CI) ^a
0	< 15	34 (47.2)	51 (38.9)		
	15 – 40	21 (29.2)	35 (26.7)	0.9 (0.5-1.6)	1.0 (0.6-1.8)
	≥ 40	17 (23.6)	45 (34.4)	0.7 (0.4-1.2)	0.8 (0.5-1.6)
0 - 50	< 15	53 (57.0)	49 (56.3)		
	15 – 40	33 (35.5)	27 (31.0)	1.1 (0.7-1.6)	1.1 (0.7-1.8)
	≥ 40	7 (7.5)	11 (12.6)	0.7 (0.3-1.6)	0.8 (0.4-1.9)
≥ 50	< 15	4 (6.5)	7 (6.3)		
	15 – 40	23 (37.1)	29 (26.1)	1.2 (0.4-3.5)	1.5 (0.5-4.6)
	≥ 40	35 (56.5)	75 (67.6)	0.9 (0.3-2.5)	1.0 (0.3-2.8)

GC = glucocorticoid

^a Adjusted for diagnosis

Figure 1. Study design. Overall analysis included all patients in the study, where subgroup analysis were conducted within the major diagnostic groups and included only patients with neoplasms, circulatory disease or respiratory diseases.

Figure 2. Distribution of the difference of absolute neutrophil and eosinophil counts between the two samples for glucocorticoid users and non-users. A: neutrophils. The vertical lines represent the cut-off value of $2 \cdot 10^9/l$ difference between the two blood samples; B: eosinophils. The vertical lines represent the cut-off value of $0.05 \cdot 10^9/l$ difference between the two blood samples.

Figure 3. Effect of the period between the two blood samples on the change in absolute neutrophil count of each patient. There is no effect of time between blood samples and the change in absolute neutrophil count. 3B is an enlargement of 3A.

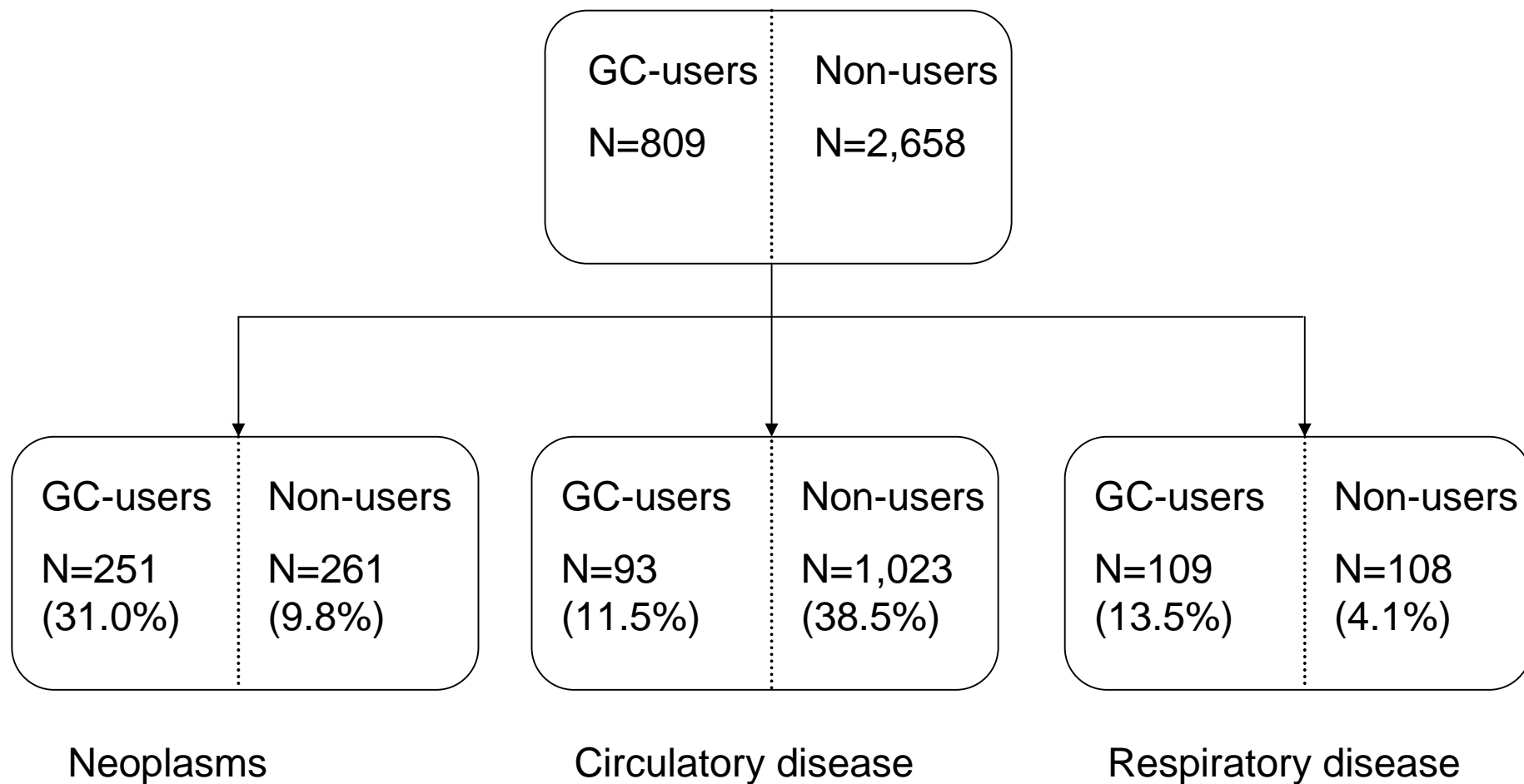


Figure 1

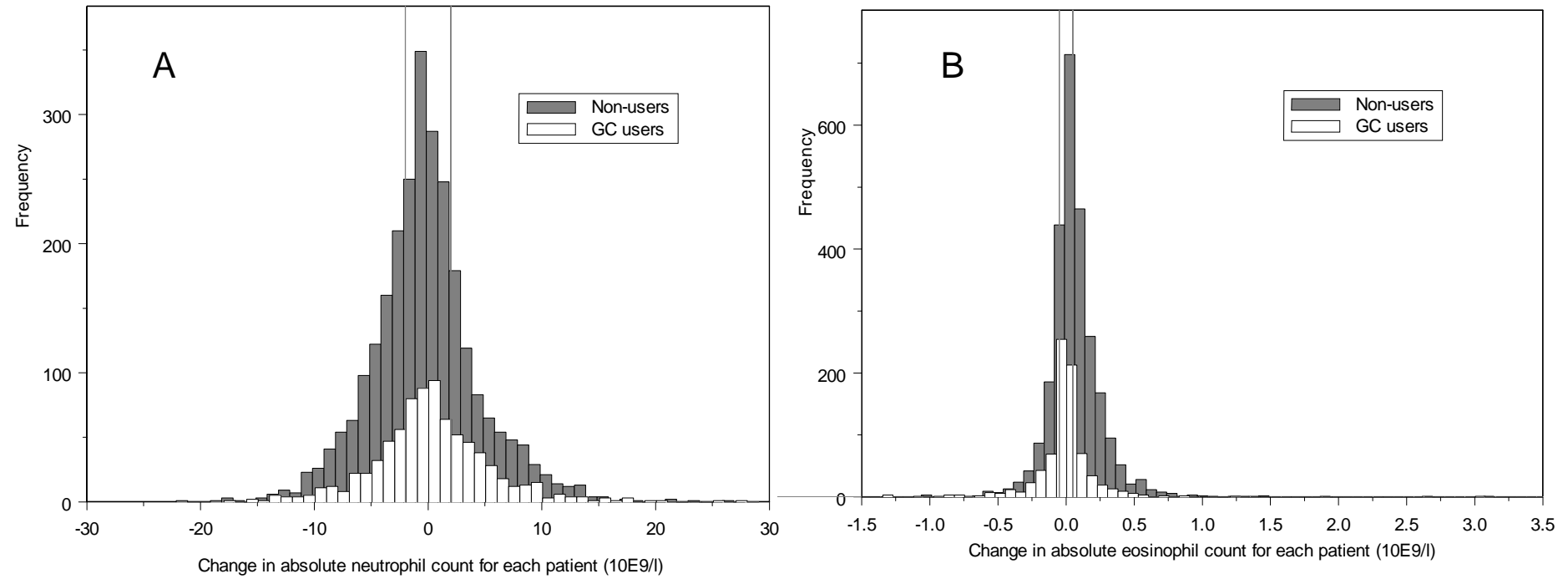


Figure 2

