Pleocytosis in Cerebrospinal Fluid Following Multiple Lumbar Punctures in Healthy Volunteers

George Atiee MD[1], John Sramek PharmD[2], Joseph Palasota MD[1], Michael Murphy MD, PhD[2], Neal R. Cutler MD[2]
[1]Worldwide Clinical Trials, Early Phase Services, San Antonio, TX, USA, [2]Worldwide Clinical Trials, Beverly Hills, CA, USA

Abstract

Introduction: Pleocytosis (white blood cell [WBC] >5/µL) in cerebrospinal fluid (CSF) is commonly observed following lumbar puncture (LP), but interpretation is often confounded by inclusion of patient populations. A better understanding of pleocytosis following LP is required due to recent interest in utilizing CNS biomarkers in psychiatric research.

Methods: Forty-one healthy male and female volunteers (aged 18-50) participated in CSF studies of potential psychotropic compounds. A cell count with differential was analyzed from the initial CSF draw at each puncture. In all studies, subjects underwent baseline LP followed by a repeat LP on Day 14. Two 2.0 mL CSF samples per time point were taken in Cryoware vials, chilled on ice, frozen and processed within 60 minutes. A two-paired T-test compared baseline to Day 14 values for statistical significance in WBCs, neutrophils and mononuclear cells.

Results: From baseline to Day 14, subjects had small but statistically significant mean increases in WBCs (2.59/µL on Day 0 vs 8.10/µL on Day 14; p = 0.0002), neutrophils (0.15/µL vs 2.59/µL; p = 0.00001), and mononuclear cells (2.44/µL vs 5.51/µL; p = 0.006). Red blood cell count was not significantly different between Day 0 and Day 14. There was no clinical evidence of infection in any subjects.

Conclusions: Statistically significant increases between baseline and Day 14 LPs in WBCs, neutrophils, and mononuclear cells were small in magnitude and not considered clinically significant. The results suggest that minor pleocytosis may occur due to residual trauma at the LP site.

Introduction

Defining Pleocytosis

Pleocytosis is defined as elevated cell count, particularly white blood cell (WBC) count, compared to normal levels in the cerebrospinal fluid (CSF). It can be a sign of injury, underlying pathology or infection. The exact WBC level required to qualify for pleocytosis remains somewhat indeterminate, as several published reviews state different WBC thresholds. For example, Straus et al [1] and Seehusen et al [2], state that normal levels of WBCs are < 5/uL. On the other hand, Deisenhammer et al [3] states a normal WBC count is < 15/uL. The composition of WBCs in normal adult CSF is also somewhat contested. Seehusen et al states that the WBC count in normal adult CSF is composed of approximately 70% lymphocytes and 30% monocytes [2], while other sources have stated normal WBC composition in CSF consists of 40-80% lymphocytes, 15-45% monocytes, and 0-6% neutrophils [4]. The type of WBCs present may be clinically relevant; CSF samples with WBC levels < 5/uL but showing 2 or more polymorphonuclear (PMN) cells are still considered abnormal [5]. Most sources generally agree that WBC levels showing clinically significant elevations or cell count differences compared to baseline may represent underlying injury or infection and warrant subsequent attention.

Literature on Pleocytosis and Lumbar Puncture

Pleocytosis is commonly reported in studies involving lumbar puncture (LP), but the contributing role of LP itself in pleocytosis has been obfuscated by the inclusion of patient populations in these studies, which may add confounding factors such as disease state, underlying injury, or contraindications that could affect the incidence and prevalence of pleocytosis. As LP is often performed as a diagnostic tool during infections or injuries which affect the brain and CNS, many studies have observed pleocytosis following LP in the context of meningitis [6] [7], focal neurological findings, febrile disorders, disturbances of consciousness, complications after head injury, neurosurgical operations [8], multiple sclerosis [9], paraneoplastic neurological syndromes [10], leptomeningeal metastases [11], hematological malignancies [12], and post-dural puncture headaches [13] [14]. Unfortunately, it has been difficult to determine what contributing factor the LP itself has towards triggering pleiocytosis in these studies, due to the potentially confounding influence of the underlying pathologies involved.

Introduction (cont.)

Several medical textbooks suggest the occurrence of pleocytosis may be a normal side effect following repeated lumbar puncture, but do not cite controlled studies in healthy subjects to validate or quantify these statements [15] [16]. In fact, only a handful of studies have examined the incidence of pleocytosis following repeated lumbar puncture in healthy subjects, and most of these studies focus exclusively on special populations. For example, a study in 1954 examined healthy children for signs of pleocytosis following repeated lumbar puncture at different time intervals, and found clinically significant increases in cell count observed in 37.5% of the samples collected within 2 days of the first puncture, 25% of the samples collected within 3-5 days of the first puncture, and 10% of the samples collected within 6-8 days of the first puncture [17]. This led them to conclude that pleocytosis within a week of an initial puncture could be caused by the puncture itself, while pleocytosis occurring > 7 days post-puncture may be due to an underlying infection.

Rationale for Present Study

The lack of current data on the incidence of pleocytosis in healthy adult subjects undergoing repeated lumbar puncture is especially relevant due to the recent interest in utilizing CNS biomarkers in psychiatric research, and the increased use of repeated lumbar puncture in healthy adult subjects volunteering for clinical studies. We sought to address this deficiency via the following study.

Methods

Subjects

Forty-one healthy adult male (n=25) and sterile or post-menopausal female (n=16) volunteers (aged 18-50, mean age 30 years) participated in several CSF studies of potential psychotropic compounds. Subjects were screened by medical history, physical exam, electrocardiogram (ECG), and routine labs, and could not have any clinically relevant abnormalities, unstable medical conditions, or any significant chronic disease, history of seizures, psychiatric disorder (including assessment with the Columbia-Suicide Severity Rating Scale), allergy, or recent hospitalization, surgery, blood or plasma donation. They also were screened to ensure they had no history of alcohol or drug abuse in the past 2 years; and no recent use of prescription or non-prescription drugs, vitamins, dietary or herbal supplements. Subjects were screened to exclude poor metabolizers of CYP2D6. Subjects were also screened to ensure that they had no conditions, surgeries, or medications that would complicate lumbar puncture; this included an X-ray of the lumbar spine to rule out any anatomical abnormalities, and a careful history for any recent febrile illnesses or infections/inflammations near the lumbar puncture site, recent use of anticoagulants, history of spinal surgery, a history of migraine or related headaches, any allergy to Betadine or Xylocaine.

Subjects were required to refrain from taking alcohol within 72 hours prior to clinic admission and to refrain from smoking 60 days prior to signing the informed consent form and also for the entire duration of their study participation. Subjects refrained from exercise from the time of the physical examination until the end of the study. Subjects fasted from 8:00 pm the night before any laboratory sample collection was planned.

Study Design

Before each study, subjects underwent a comprehensive screening from Days -22 to Day -2. Written informed consent was obtained from all subjects (or their guardian or legal representative, as applicable for local laws). Anatomic abnormalities in the lumbar spine were evaluated by X-ray during screening to rule out absolute or relative anatomic contra-indication to LP at the investigator's discretion. The use of a fluoroscope to assist in the procedure was at the investigator's discretion with the consent of the subject. In all studies, subjects who met the inclusion criteria and did not fulfill any of the exclusion criteria underwent a single baseline (Day 0) LP followed by a repeat LP on Day 14. A cell count with differential was analyzed from the initial CSF draw at each puncture (baseline and Day 14). Two 2.0 mL CSF samples per time point were taken in Cryoware vials, chilled on ice, frozen and processed within 60 minutes. Subjects remained in the trial center through Day 17. A safety follow-up telephone contact was performed approximately on Day 44.

Statistical Analysis

A two-paired T-test compared baseline to Day 14 values for statistical significance in RBCs, WBCs, neutrophils and mononuclear cells.

Results

As shown in **Table 1,** from baseline to Day 14 subjects had small but statistically significant mean increases in WBCs (2.59/µL on Day 0 vs 8.10/µL on Day 14; p = 0.0002), neutrophils (0.15/µL vs 2.59/µL; p = 0.00001), and mononuclear cells (2.44/µL vs 5.51/µL; p = 0.006). Red blood cell count was not significantly different between Day 0 and Day 14.

Table 1: Subject CSF Cell Counts Post LP on Day 0 and Day 14

	WBC (/µL)		RBC (10^3/µL)		Neutrophil (/µL)		Mononuclear (/µL)	
	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14	Day 0	Day 14
Sample size:	41	41	41	41	41	41	41	41
Mean:	2.59	8.10	0.17	0.22	0.15	2.59	2.44	5.51
Standard Deviation:	1.48	8.61	0.59	0.57	0.36	3.22	1.42	6.57
Minimum:	1	1	0	0	0	0	1	1
Maximum:	7	45	3	2	1	19	7	36
P-value (2-paired T-Test):	0.0002		0.6999		0.00001		0.006	

There was no clinical evidence of infection in any subjects. In addition, the majority of the cell count differences between baseline and Day 14 for all cell categories was very small, with most subjects showing absolute cell count differences between the two LPs hovering around zero (**Figures 1, 2 and 3**).

Figure 1: Subject Percent Distribution of WBC Cell Count Difference between Baseline and Day 14

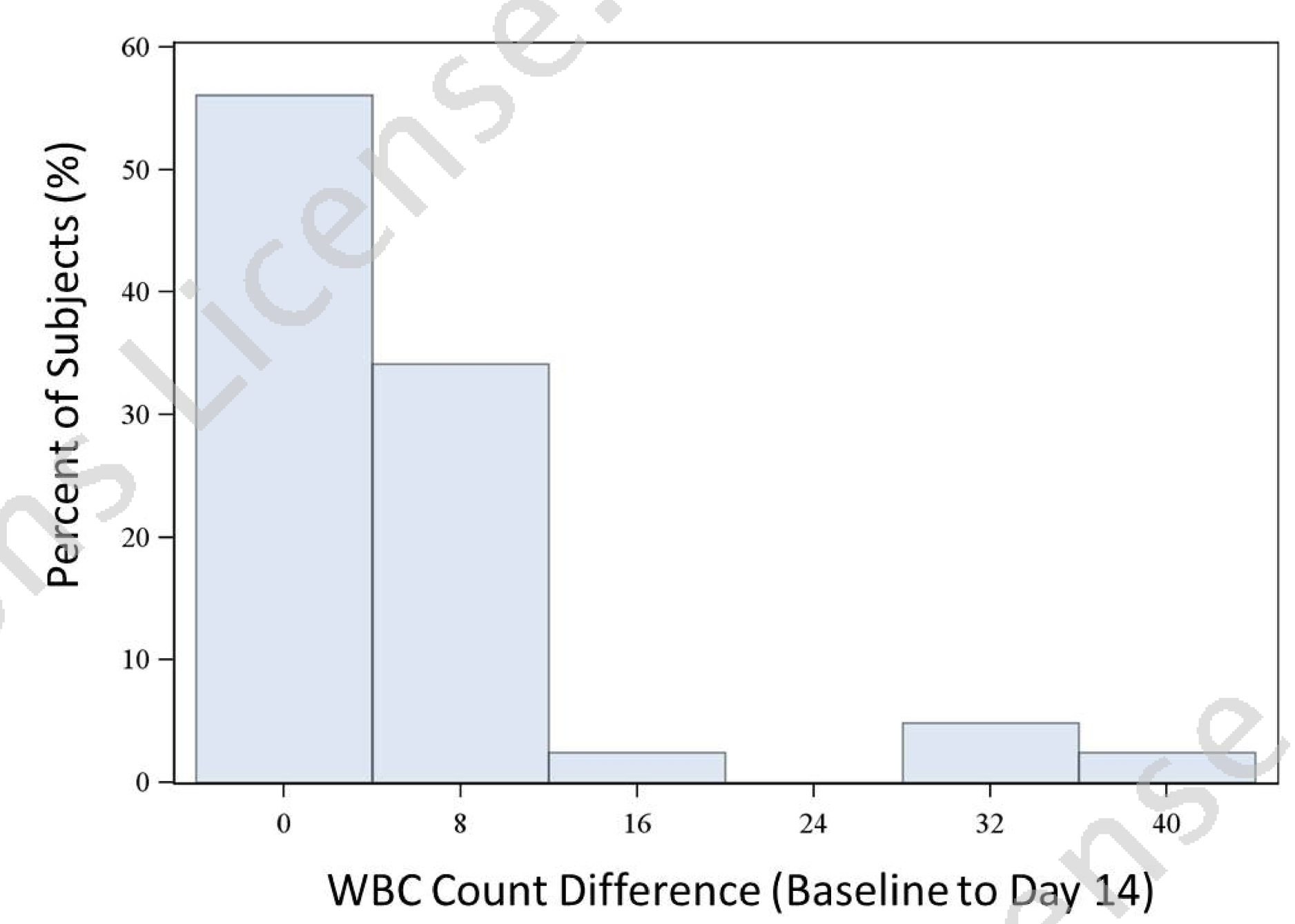
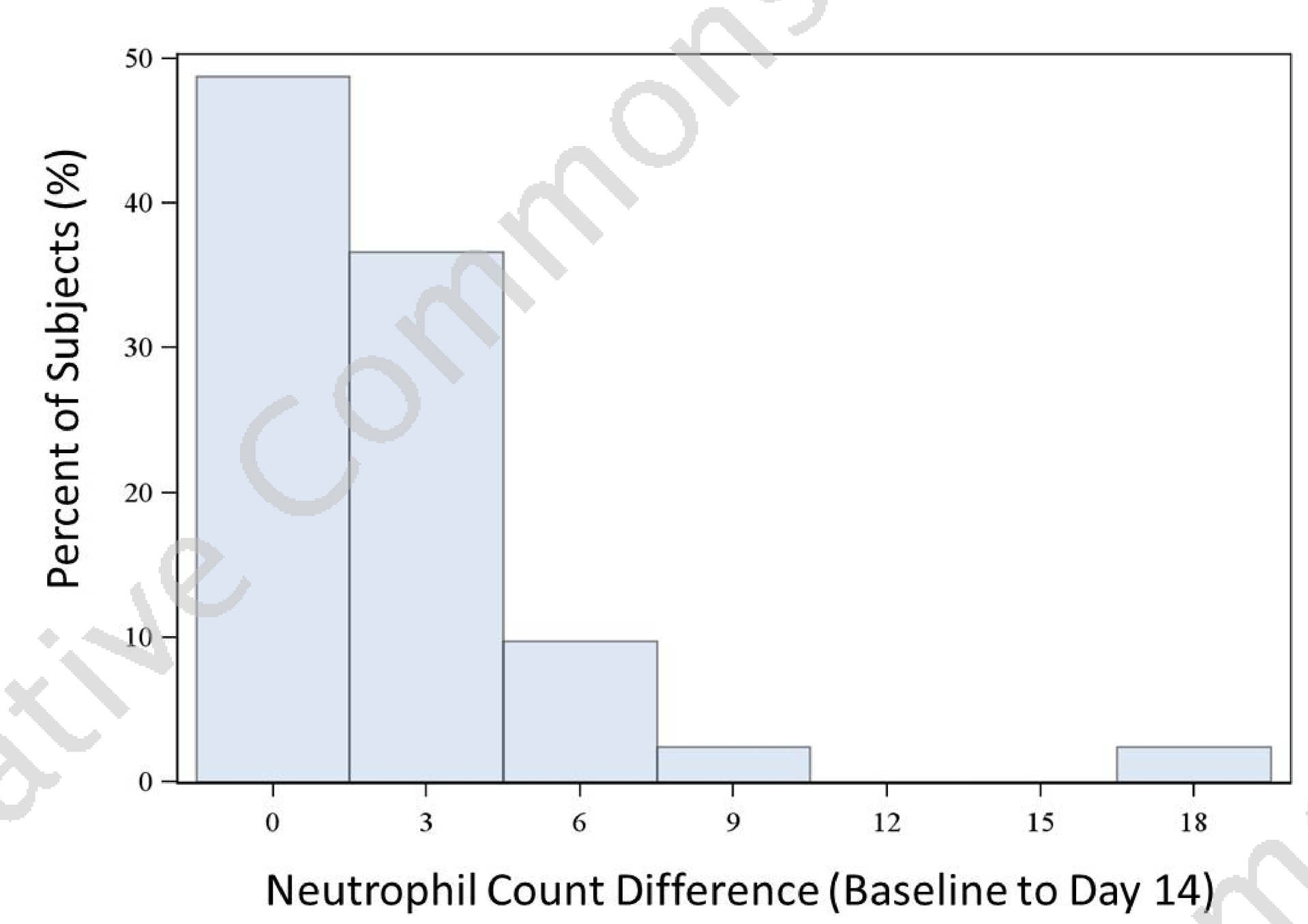
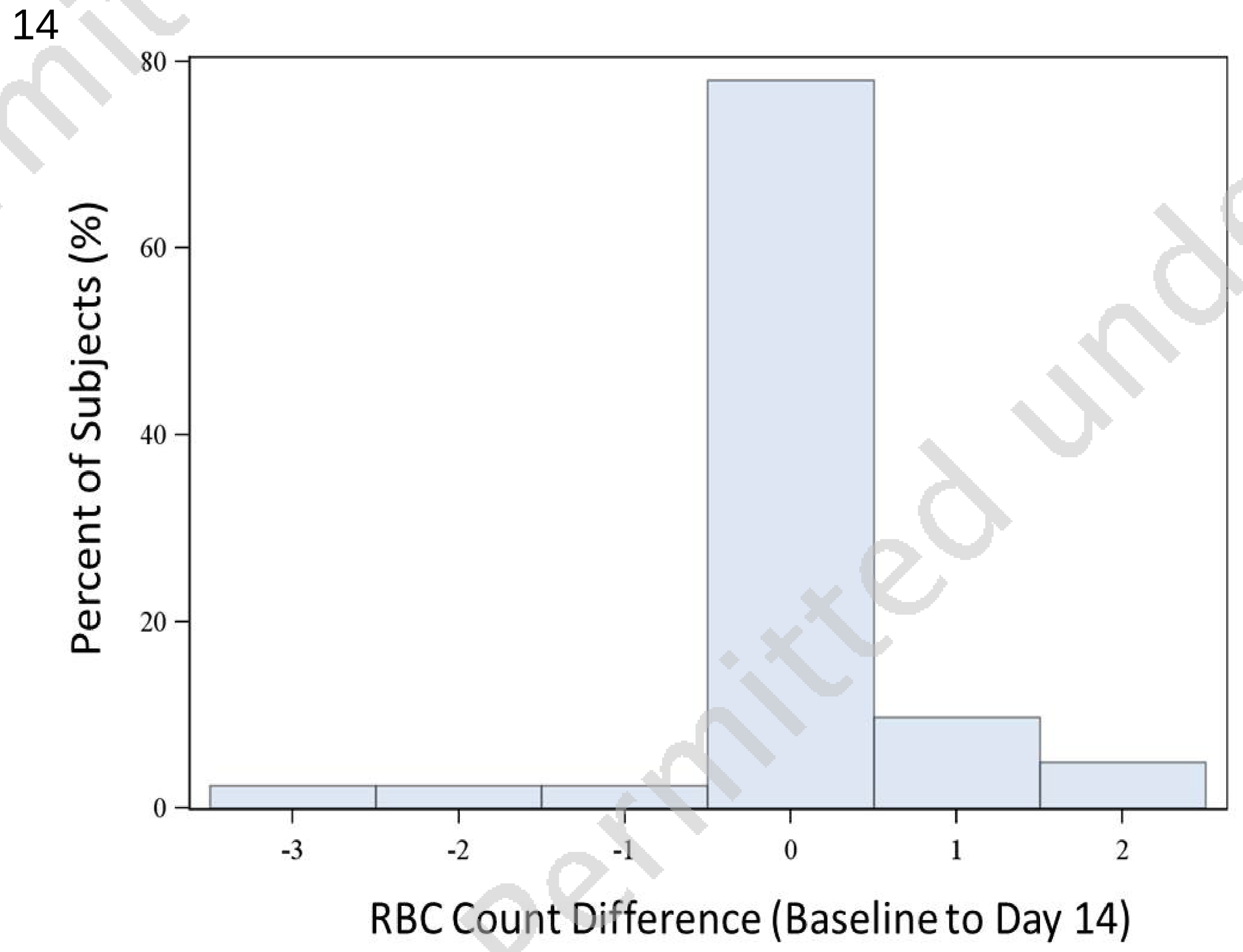


Figure 2: Subject Percent Distribution of Neutrophil Cell Count Difference between Baseline and Day 14



Results (cont.)

Figure 3: Subject Percent Distribution of RBC Cell Count Difference between Baseline and Day 14



Conclusions

This study evaluated the incidence of pleocytosis with repeated lumbar puncture over a 14 day period in healthy adult volunteers. The use of healthy adult volunteers in this study presents a clearer picture of pleocytosis than previous studies that have used patient populations or specialized populations, by removing confounding factors such as disease state, age, injury, or contraindications which may affect the incidence and prevalence of pleocytosis during LP. Statistically significant increases between baseline and Day 14 LPs in WBCs, neutrophils, and mononuclear cells were small in magnitude and not considered clinically significant. There were no statistically significant changes in RBCs between baseline and Day 14. The results suggest that minor pleocytosis may occur due to residual trauma at the LP site with repeated lumbar puncture, but it is not severe nor a cause for major concern if the subject is otherwise healthy. Large deviations in CSF cell count levels should be followed up with, especially if infection or injury is suspected and other clinically significant signs or symptoms are present.

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