Diurnal and Racial Variance of White Blood Cell Parameters in Early Phase Clinical Trials: A Retrospective Analysis of Pooled Data from Multiple Phase I Trials

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Background

White Blood Cell (WBC) counts are a common subject inclusion criterion for clinical trials as they are indicators of immunocompetence, infection, and inflammation in individuals.

Genetic variation between ethnic groups modulates the abundance of WBC subpopulations. Genome wide association studies have shown that the genetic loci associated with the Duffy antigen receptor for chemokines (DARC) –(rs2814778)- is associated with a lower neutrophil count in people of African ancestry [1-3].

WBC count is also strongly influenced by circadian factors. Neutrophil and monocyte migration from blood to tissues showed circadian oscillations that cause variations in the magnitude of inflammatory responses [4-5] and the diurnal variation of lymphocyte trafficking appears to be under adrenergic control [6].

Reference Intervals (RIs) for common clinical laboratory tests are usually not developed separately for different subpopulations despite the recognized ethnic differences in RIs for various laboratory tests.

This study assessed the interaction of ethnicity and time of day on the White Blood Cell count. Additionally, this study aimed to address the question on whether the RIs of common laboratory tests should be different between different ethnic groups and to propose data driven in- and exclusion criteria for clinical trials that fit a healthy subject population.

References

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Methods

This study analysed pooled data from 35 clinical trials conducted over a period of seven years (January 2010-January 2017) at Richmond Pharmacology, St George's, University of London, UK. All subjects provided written informed consent for that specific clinical trial and all the 35 trials were approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and a Research Ethics Committee and performed in accordance with the guidelines established by the declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines.

Results from 13,332 venous blood samples, from 7157 healthy subjects (2668 women and 4489 men) aged 18 to 76 years of age were obtained at screening and admission prior to exposure to any study related medication. Patient studies were excluded. The anonymised white blood cell count data obtained from these samples and used in this research was done so in accordance with the Medical Research Council's advice on the use of anonymised data [7].

Samples were obtained via venepuncture using a standardised aseptic technique by trained staff and were obtained throughout the day. Cell counts were carried out within five hours by an accredited pathology laboratory (The Doctors Laboratory Limited, London, UK - TDL).

Table 1: Baseline characteristics of subjects.

		Ethnicity		
Variable	**Statistics**	**Black**	**Non-black**	**All**
	n	926 (13%)	6231 (87%)	7157 (100%)
Age	mean (SD)	30.29 (10.1)	31.40 (11.2)	31.26 (11.1)
Sex	Female (% of total)	410 (6%)	2258 (32%)	2668 (37%)
	Male (% of total)	516 (7%)	3973 (56%)	4489 (63%)
Fasting	Fed (% of total)	27 (0.4%)	249 (3%)	276 (4%)
	Fasted (% of total)	899 (13%)	5982 (84%)	6881 (96%)

Statistical analysis

The data obtained from the haematological investigations of the subjects' samples were grouped by their self-ascribed Race into the following groups: Black (Black African & Black Caribbean) and non-Black (Caucasian and Asian) as no relevant differences between Asian and Caucasian were found. Their self-ascribed "race" was ascertained at the initial telephone recruitment stage.

Mean values and standard deviations per time of day for each racial group for the following haematological parameters were calculated; Total White Cells, Basophils, Eosinophils, Lymphocytes, Monocytes, and Neutrophils.

The haematological parameters were analysed by generalised estimating equation (GEE) models in which age, gender, fast status, race, time, and interaction between time and race were treated as fixed effects, the subjects as a cluster effect. The estimated between-race and within-race differences in least squares means (marginal means) from the GEE models are therefore derived and reported together with their 95% confidence intervals. Reported *P*-values are two-sided and a *P* value of <0.05 was considered statistically significant. Adjusted normal ranges of haematological parameters were calculated for black and non-blacks and in samples collected in the morning vs samples collected in the evening. All statistical analyses were carried out by using the Statistical Analysis System (SAS) version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

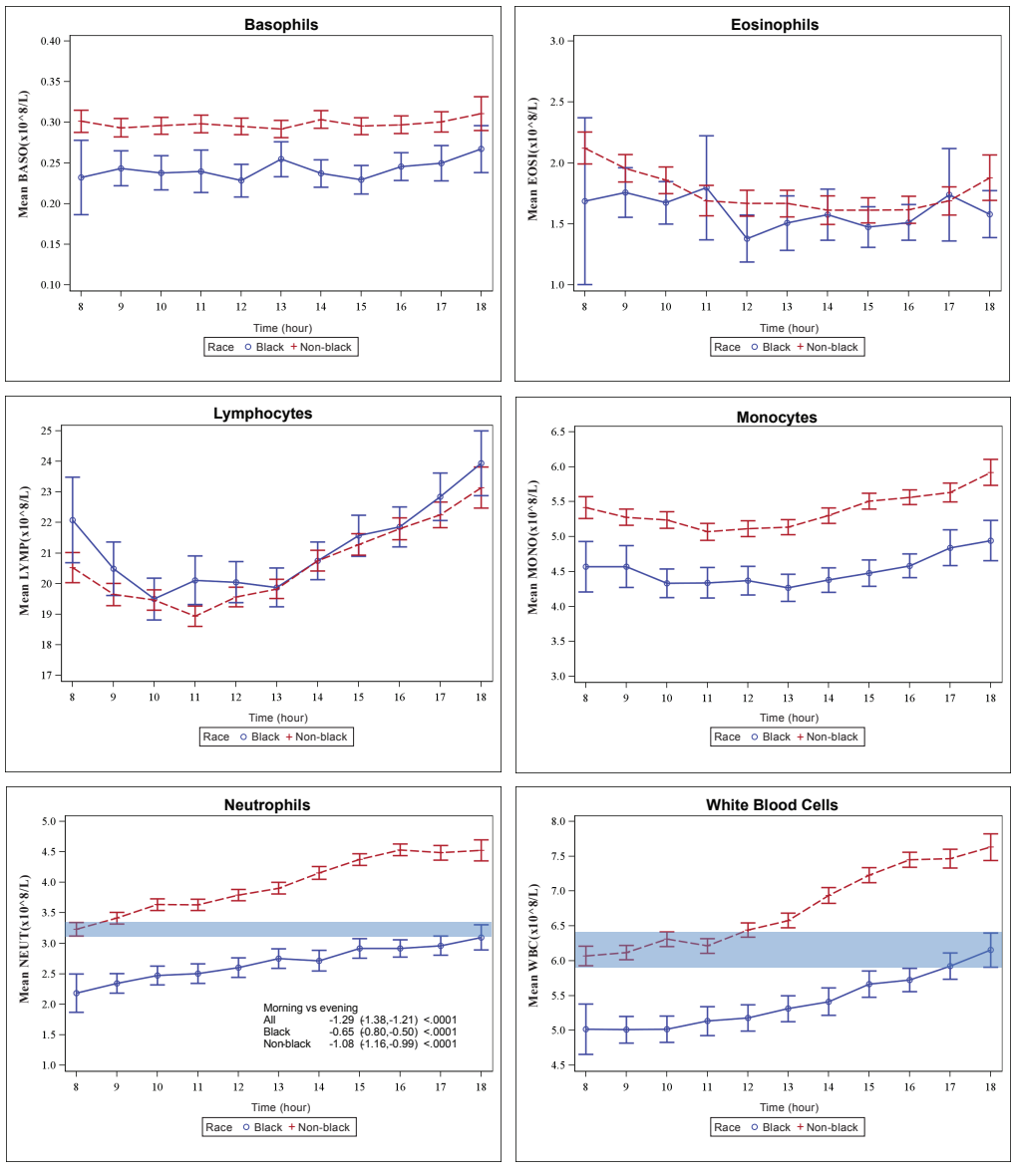

Figure 1: Graphs showing Least Square means of different blood cells by time and race.

Table 2: Ethnic and diurnal differences.

Eosinophils	Basophils	Monocytes	Lymphocytes	Neutrophils	WBC
• No significant differences between ethnic groups. • No significant differences over time.	• Black have a lower basophil count than non-black people. • Constant throughout the day. • Mean of non-black is +20% from black without overlap with the exception of one timepoint.	• Black have a lower basophil count than non-black people. • Constant throughout the day. • Mean of non-black is +20% from black without overlap with the exception of one timepoint.	• No difference between black and non-black • Evening values are significantly higher than mid-day values. • Mean morning values also appear higher.	• Black have a significantly lower counts than non-black people (only a small overlap). • Evening values are significantly higher p<0.0001 than morning.	• Black have a lower count than non-black people (only a small overlap). • Evening values are significantly higher than morning.

Table 3: Proposed Reference Interval. TDL's reference ranges are reflective of standard haematology reference intervals used in NHS hospitals, private clinics and quoted by Haematology charities across the UK.

Leukocyte	Ethnicity	Morning		Evening		TDL Reference Range
WBC(x10^9/L)	**Black**	**2.6**	**7.5**	**2.8**	**8.8**	3.0-10.0
	Non-black	3.0	9.1	3.8	10.9	
NEUT(x10^9/L)	**Black**	**0.5**	**4.3**	**0.5**	**5.4**	**2.0**-7.5
	Non-black	0.9	5.8	1.5	7.4	
LYMP(x10^9/L)	**Black**	**0.9**	**2.9**	**1.0**	**3.3**	1.2-3.7
	Non-black	0.9	3.0	1.0	3.4	
MONO(x10^9/L)	**Black**	**0.1**	**0.8**	**0.2**	**0.8**	0.2-1.0
	Non-black	0.2	0.9	0.2	0.9	
EOSI(x10^9/L)	**Black**	**0.0**	**0.9**	**0.2**	**0.5**	0.0-0.4
	Non-black	0.0	0.6	0.2	0.6	
BASO(x10^9/L)	**Black**	**0.0**	**0.1**	**0.0**	**0.1**	0.0-0.1
	Non-black	0.0	0.1	0.0	0.1	

Conclusions

- Our data is well aligned with literature.
- We could show significant WBC differences between black and other ethnicities
- This is largely driven by the different neutrophil count
- There is a large diurnal difference between morning and evening (when counts are higher)
- The diurnal variation is of the same extent between black and non-black
- An ethnicity specific reference range allowing a more proportionate inclusion of black people into trials may be warranted
- Limitations of this study are:
  - The demographic is weighed towards white and Asian males
  - Data was derived from samples taken during "normal" working hours only.

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