

# ORAL PRESENTATION



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

EUFEMED, LYON

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# WHY DID WE DO THIS ?



## What we already know about this topic:

- There is a diurnal rhythmicity of blood cell populations.
  - Lymphocytes and monocytes show peak concentrations times during the night.
  - Neutrophil/monocyte migration shows circadian oscillations
- Individuals of African origin have a lower white blood cell and absolute neutrophil count (benign ethnic neutropenia).
  - BEN occurs in <1% of individuals of European ancestry and is diagnosed in up to 30% of individuals of African ancestry.
  - Condition linked to a mutation in the DARC gene – duffy null allele.

# WHY DID WE DO THIS ?



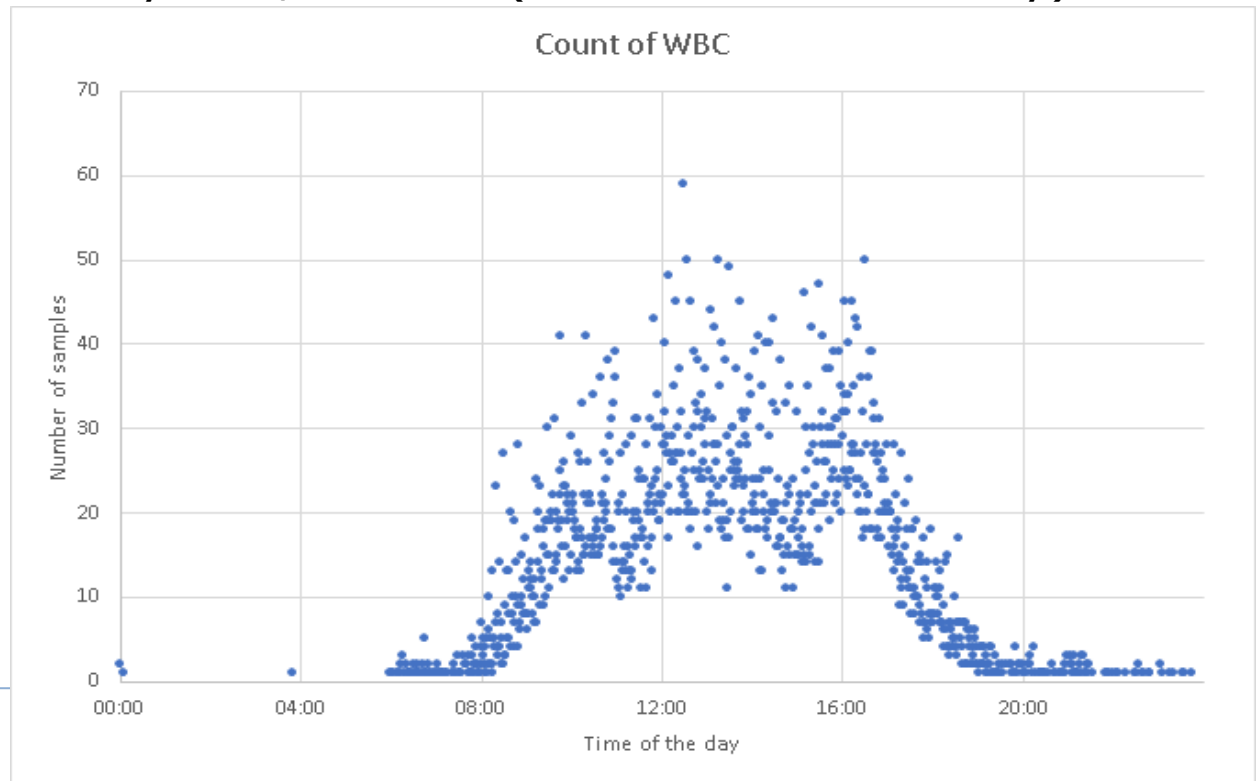
1. The count variation over the day is likely to disadvantage potential trial participants belonging to ethnic groups with lower baseline WBC count.
2. We wanted to assess the differences between readings from our centre vs. reports in literature on
  - a. Diurnal variations
  - b. Ethnic differences
3. Assess whether the normal values from our central lab “fit”
4. Propose data driven inclusion/exclusion criteria for clinical trials that fit a diverse, healthy subject population

# METHODS



This study analysed pooled data from our clinical trial database. **We selected 35 clinical trials conducted over a period of 7 years** (2010-2016 inclusive). **Patient studies were excluded.** All studies were run at Richmond Pharmacology, St George's, University of London. All samples were analysed by TDL, London (our central laboratory).

**13,332 blood samples** from **7157 individuals** samples obtained at screening and admission **prior to exposure** to any study related **medication**



# DEMOGRAPHICS



**Table 1 Baseline characteristics of patients**

		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%)

**No relevant differences between Asian and Caucasian were found; therefore data for Asian and Caucasian has been pooled to “non-black”.**

# DATA ANALYSIS



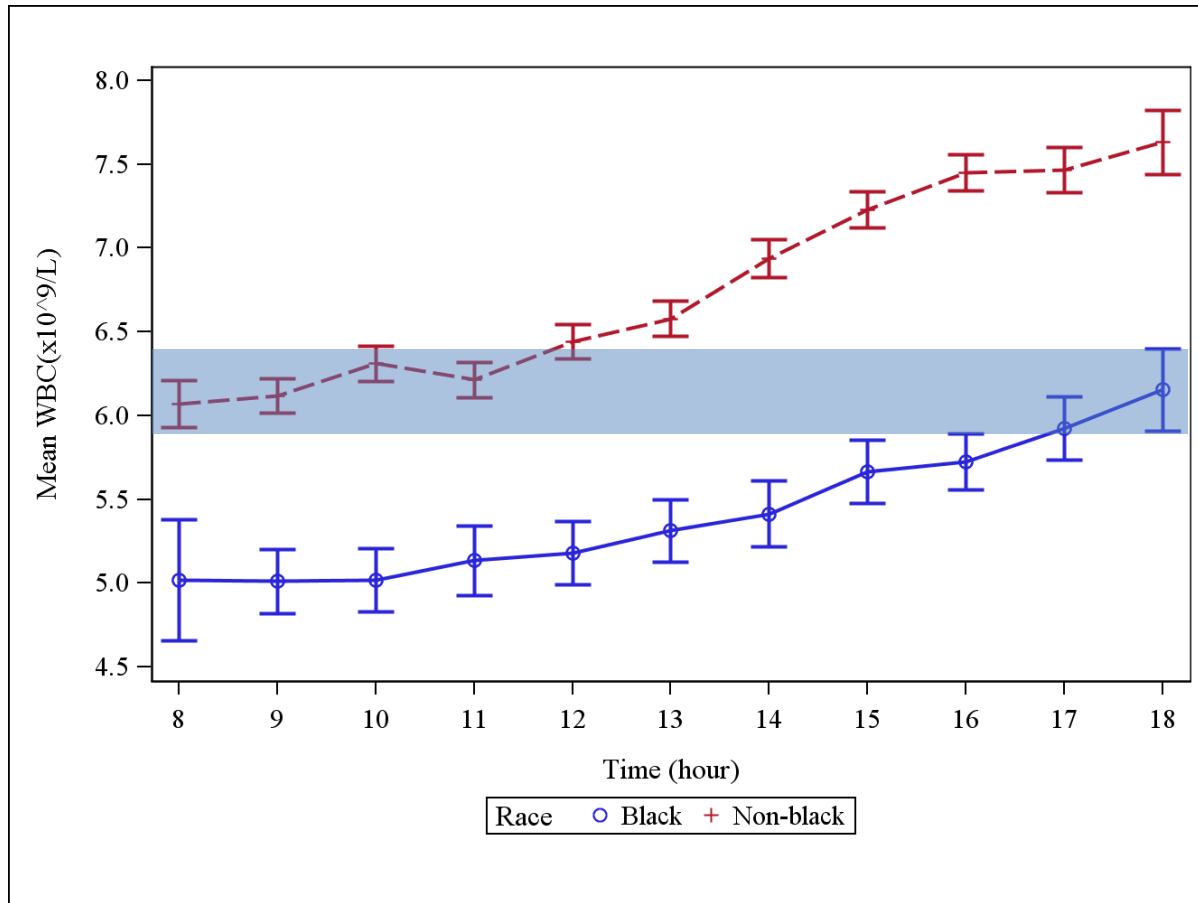
The haematological parameters were analysed by mixed mode generalised estimating equation (GEE) models in which age, sex, fast status, race, time, and interaction between time and race were treated as fixed effects, the subject as a cluster effect.

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.

**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 Duolao Wang Department of Clinical Sciences, Liverpool School of Tropical Medicine using the Statistical Analysis System (SAS) version 9.3 (SAS Institute, Inc., Cary, NC, USA).

# WHITE BLOOD CELL COUNT



## Differences

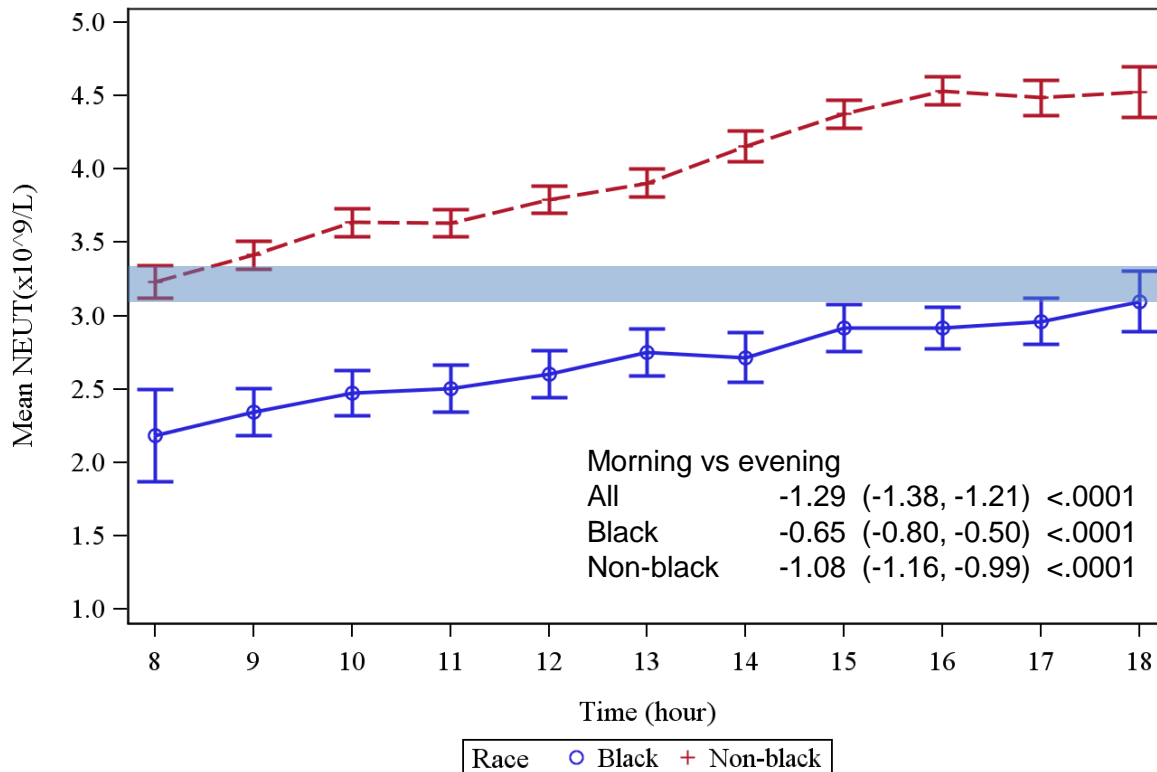
- Ethnicity
  - Black have a lower WBC count than non-black people
- Diurnal
  - Evening values are significantly higher than morning
- Only small overlap
  - Normal reference?



# NEUTROPHIL COUNT



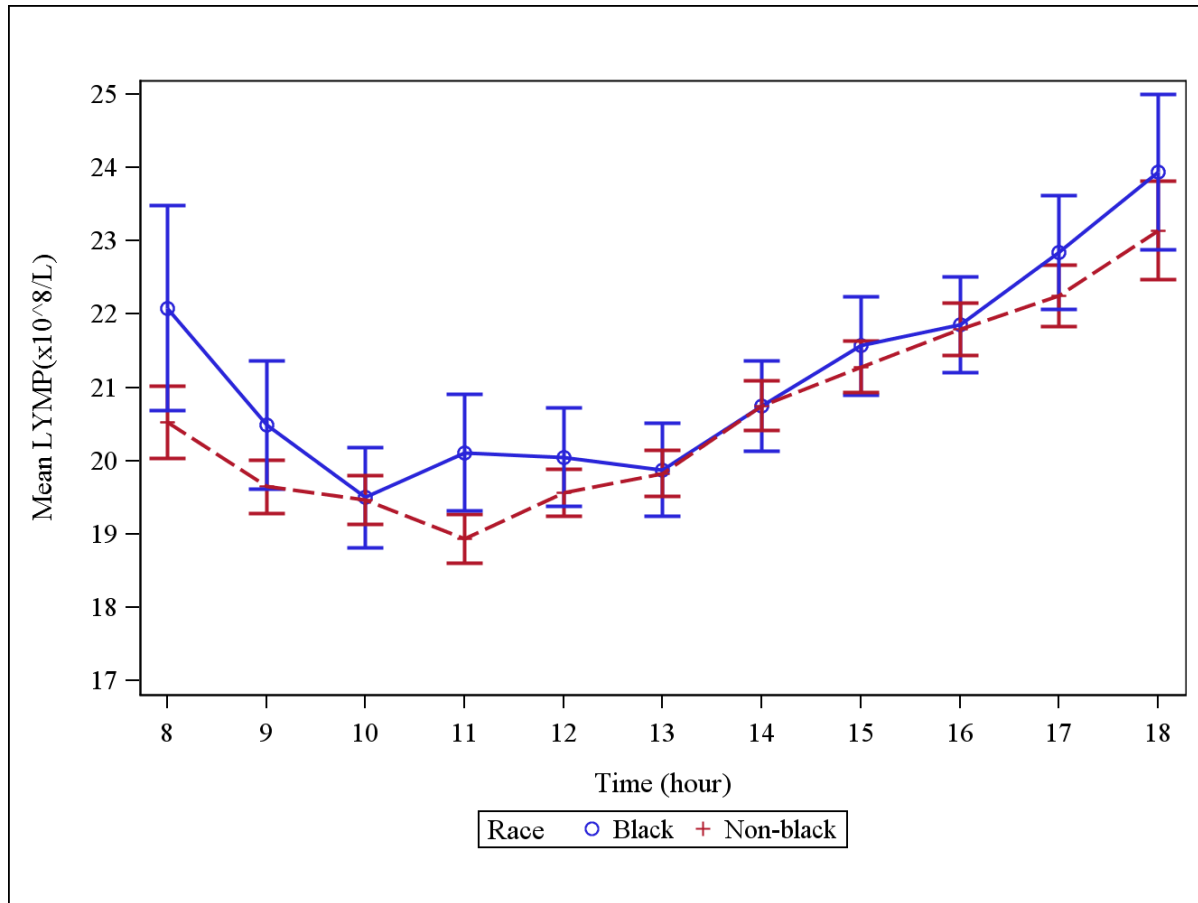
Figure 1.5: Mean NEUT value by time and race



## Differences

- Ethnicity
  - Black people have a significantly lower neutrophil counts than non-black people
- Diurnal
  - Evening values are significantly higher  $p < 0.0001$  than morning

# LYMPHOCYTE COUNT



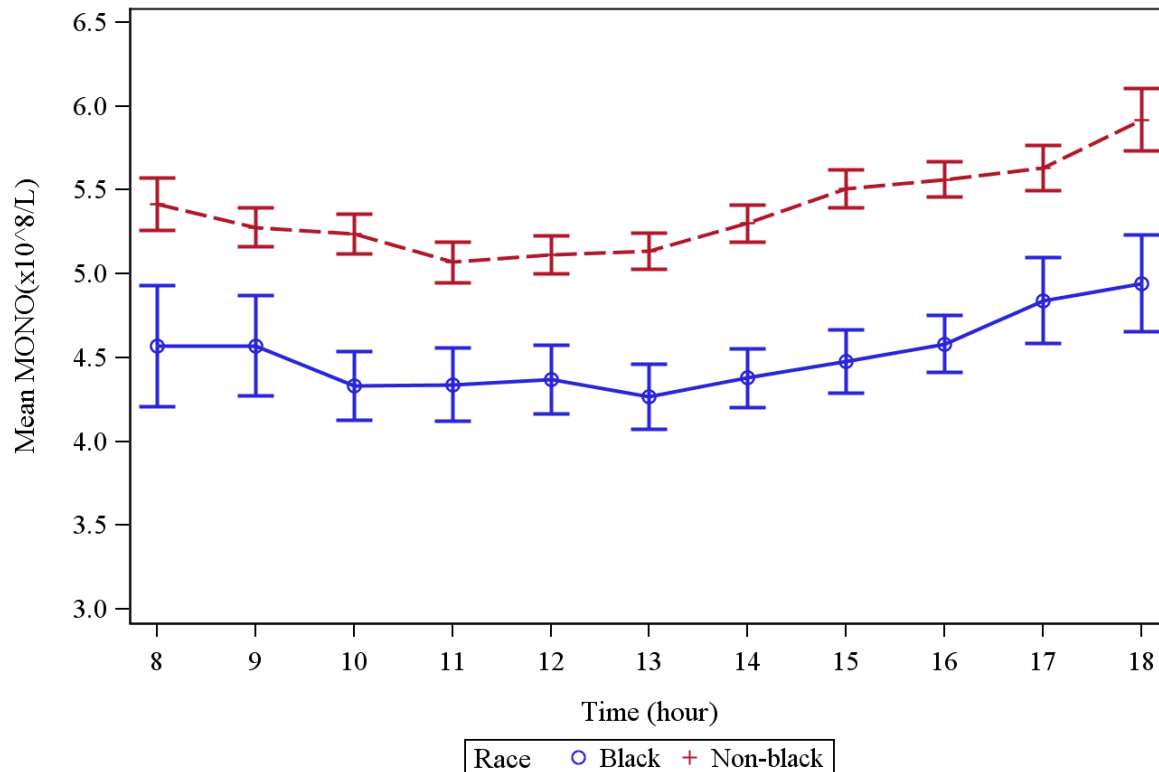
## Differences

- Ethnicity
  - No difference between black and non-black
- Diurnal
  - Evening values are significantly higher than mid-day values
  - Mean morning values also appear higher

# MONOCYTE COUNT



Figure 1.4: Mean MONO value by time and race

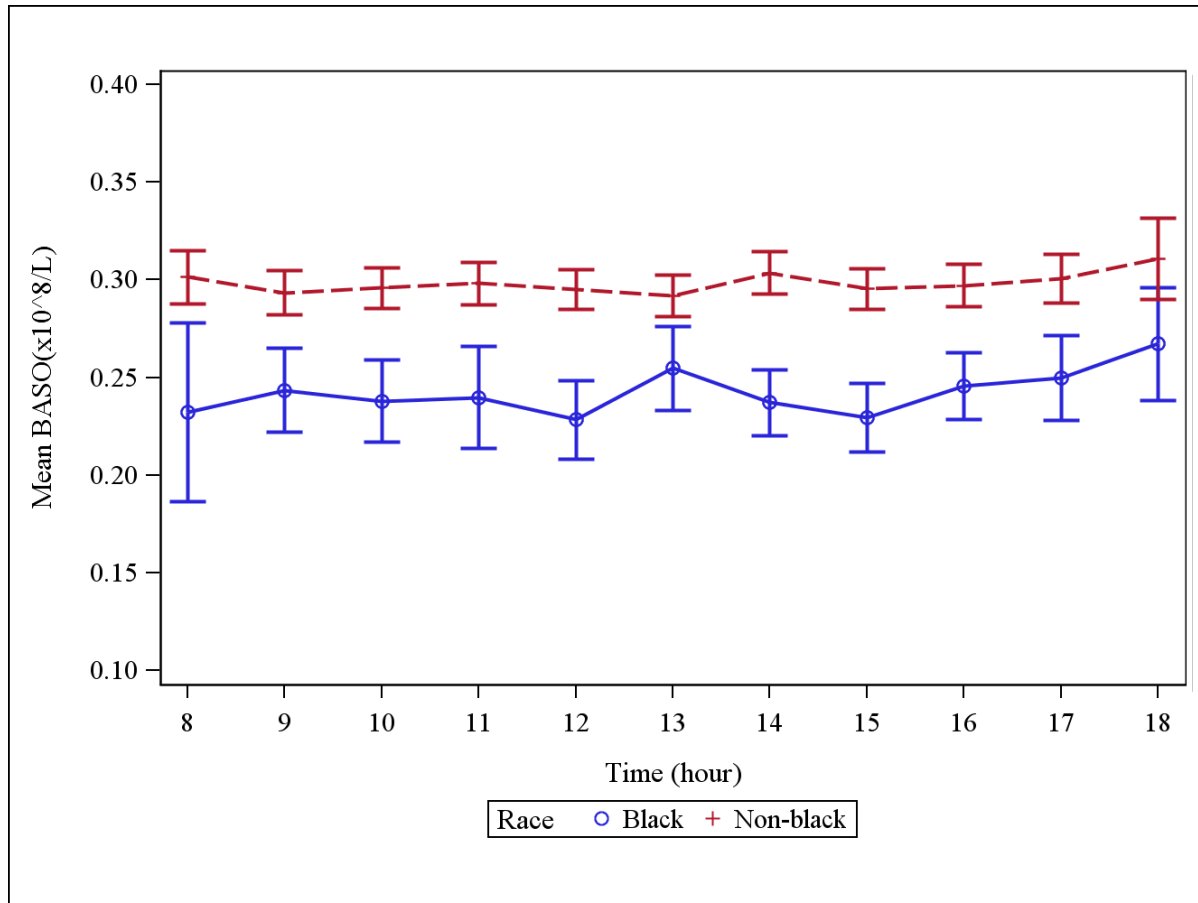


## Differences

- Ethnicity
  - Black have a lower monocyte count than non-black people
  - Constant throughout the day
- No overlap
  - Mean of non-black is +20% from black without overlap with the exception of one timepoint



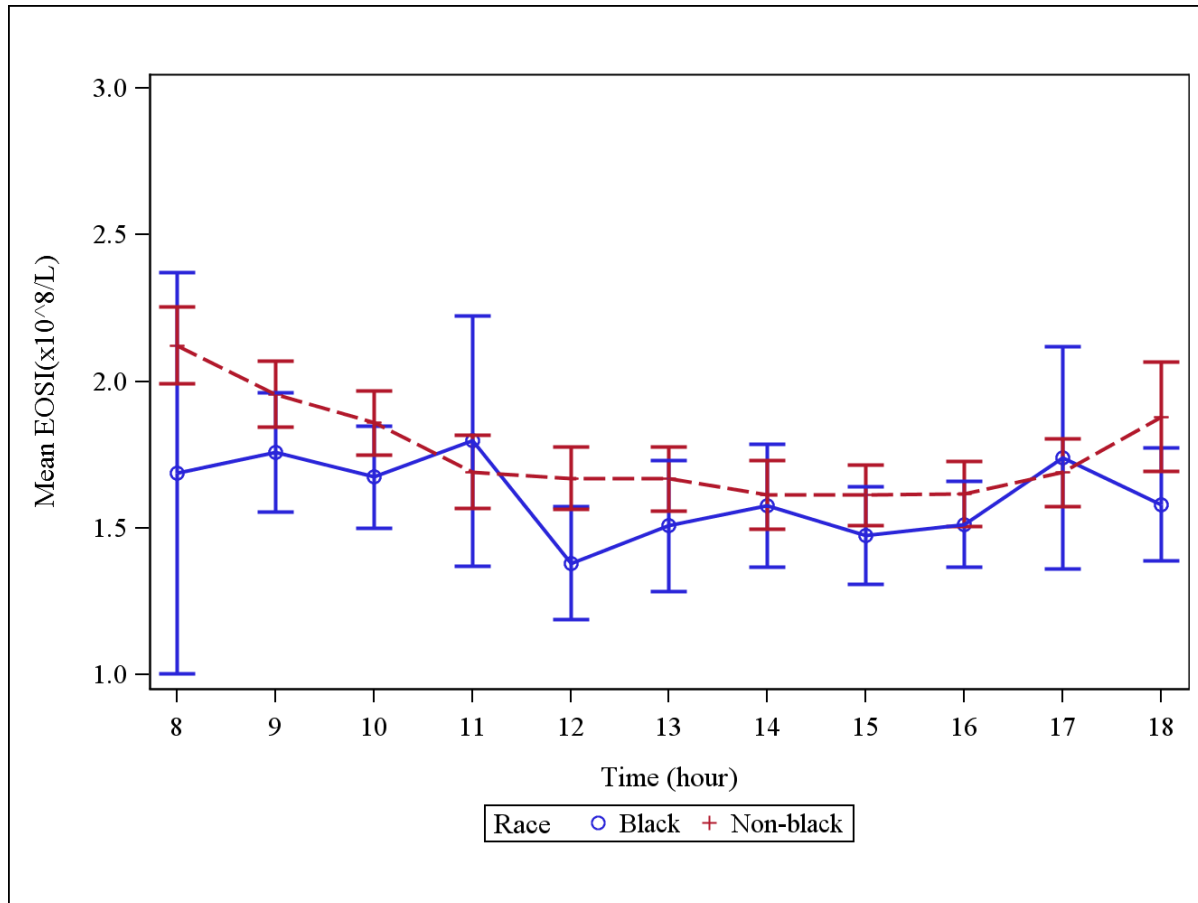
# BASOPHIL COUNT



## Differences

- Ethnicity
  - Black have a lower basophil count than non-black people
  - Constant throughout the day
- No overlap
  - Mean of non-black is +20% from black without overlap with the exception of one timepoint

# EOSINOPHIL COUNT



## Differences

- Ethnicity
  - No significant differences
- Diurnal
  - No significant differences



# PROPOSED REFERENCE RANGE



Leukocyte	Ethnicity	Morning	Evening	TDL Reference Range
WBC(x10 <sup>9</sup> /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 <sup>9</sup> /L)	Black	<b>0.5</b> 4.3	<b>0.5</b> 5.4	<b>2.0-7.5</b>
	Non-black	<b>0.9</b> 5.8	<b>1.5</b> 7.4	
LYMP(x10 <sup>9</sup> /L)	Black	<b>0.9</b> 2.9	<b>1.0</b> 3.3	1.2-3.7
	Non-black	<b>0.9</b> 3.0	<b>1.0</b> 3.4	
MONO(x10 <sup>9</sup> /L)	Black	<b>0.1</b> 0.8	0.2 0.8	0.2-1.0
	Non-black	0.2 0.9	0.2 0.9	
EOSI(x10 <sup>9</sup> /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 <sup>9</sup> /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:
  - Significant WBC differences between black and non-black racial groups - largely driven by the different neutrophil count
  - There is a big diurnal difference between morning and evening (when counts are higher)
  - The diurnal variation is of the same extent between black and non-black groups



# CONCLUSIONS



- An ethnicity- and time-specific reference ranges may help healthy volunteer clinical trials by:
  - allowing a more proportionate inclusion of black people
  - better reflecting the normal WCC parameter counts for both groups in healthy volunteers

Additionally:

- aiding the development of rules for post-dose changes
- facilitating more accurate and clinically relevant post-dose safety monitoring and reporting



# QUESTIONS



**Thank you!**



# QUESTIONS



**Thank you!**



# NORMAL REFERENCE RANGES



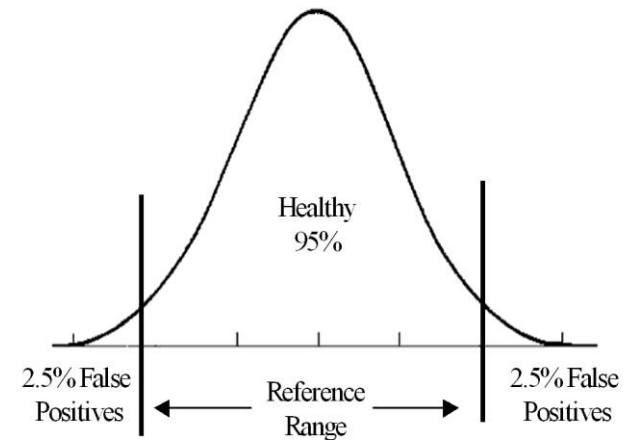
Clinical trials protocols base exclusions of participants on the “normal laboratory reference range”

Table. Reference values of 10 common blood chemistry analytes of healthy population (age; 0-80 years) of Rawalpindi-Islamabad area. Study conducted by AFIP Rawalpindi during 1993-94 (n=2115).

Analytes (Units)	Sex	Newborn (age:0)	Children (1-10 yrs)	Children (11-14 yrs)	Adults (15-40 yrs)	Adults (41-60 yrs)	Geriatric (61-80 yrs)
Glucose (mmol/L)	M	3.1-5.7	3.1-5.8	3.5-6.0	3.6-6.0	3.7-6.3	3.4-5.2
	F	3.1-5.7	3.2-5.9	3.6-6.0	3.5-5.9	3.6-6.2	3.1-6.1
Cholesterol (mmol/L)	M	2.5-5.1	2.8-5.6	3.0-5.9	3.2-6.6	3.3-6.7	3.1-6.4
	F	2.5-5.1	2.7-5.5	2.8-5.8	3.1-6.4	3.5-6.9	3.2-6.5
Triglycerides (mmol/L)	M	0.3-2.0	0.45-2.1	0.5-2.2	0.6-2.3	0.55-2.25	0.45-2.1
	F	0.3-2.0	0.4-2.05	0.45-2.2	0.55-2.2	0.6-2.2	0.4-2.0
Urea (mmol/L)	M	2.4-5.2	2.6-5.8	2.7-6.2	2.8-6.4	3.0-6.8	3.2-6.9
	F	2.4-5.2	2.5-5.7	2.6-6.1	2.7-6.3	2.9-6.5	3.1-6.8
Creatinine (umol/L)	M	55-120	33-80	59-128	65-132	68-138	70-142
	F	55-120	31-78	50-110	54-119	57-120	58-125
Uric acid (umol/L)	M	135-390	125-351	150-410	164-430	174-450	167-435
	F	135-390	120-344	126-375	132-380	137-385	133-375
Bilirubin, total (umol/L)	M	14-52	4-17	5-18	4-17	5-16	4-17
	F	14-52	4-16	4-17	3-15	4-15	3-16
Protein, total (g/l)	M	55-73	56-79	57-83	58-82	59-83	58-80
	F	55-73	55-80	56-82	58-80	58-81	56-78
Alanine transaminase (U/L)	M	20-65	16-52	15-45	11-42	11-41	10-40
	F	20-65	14-49	13-43	10-41	9-40	10-40
Alkaline phosphatase (U/L)	M	206-630	186-690	185-620	126-297	101-287	105-280
	F	206-630	191-706	175-610	107-290	103-292	108-285

... however ...

reference ranges vary and are arbitrary



# BACKGROUND



## ALREADY KNOWN ABOUT THIS SUBJECT

- Individuals of African origin have a lower white blood cell and absolute neutrophil count (benign ethnic neutropenia).
- The difference seen between ethnicities is due to a common African-derived null-variant of the Duffy antigen receptor for chemokines (DARC) gene.
- There is a diurnal rhythmicity of blood cell populations.
- Lymphocytes and their subpopulations and monocytes show peak concentrations times during the night.

## THIS STUDY ADDS

- Black individuals have lower neutrophil and whole blood cell counts than Caucasian and Asian.
- No difference between Japanese and Caucasian Healthy volunteers was found
- Significant diurnal differences in WBC, neutrophil, monocyte counts, between black and non-black people.
- A considerable number of the people we studied fall outside the lower limit of the normal reference range provided by our central laboratory for total WBC, neutrophils, lymphocytes, and monocytes.

# Discussion



## Nonchemotherapy

- Clozapine
- Dapsone
- Hydroxychloroquine
- Infliximab
- Lamotrigine
- Methimazole
- Oxacillin
- Penicillin G
- Procainamide
- Propylthiouracil
- Quinidine/Quinine
- Rituximab
- Sulfasalazine
- Trimethoprim-sulfamethoxazole
- Vancomycin

## Chemotherapy

- Alkylating agents
- Anthracyclines
- Antimetabolites
- Camptothecins
- Epipodophyllotoxins
- Hydroxyurea
- Mitomycin C
- Taxanes
- Vinblastine

- Benign hereditary leukopenia-neutropenia has been reported in several ethnic groups, including Yemenite Jews, Blacks of South African extraction, West Indians and Arab Jordanians.
- The subjects with BL were shown not to have an increased incidence of infections, and
- their response to infection did not differ from subjects having normal white blood cell counts.
- Severe neutropenia resulting in neutropenic fever and infection can lead to hospitalization, the need for broad-spectrum antibiotics, and the potential sequelae of bacteraemia, and it can be fatal.



# LIMITATIONS



- The demographic is weighed towards white and Asian males
- Data is derived from samples taken during “normal” working hours only

