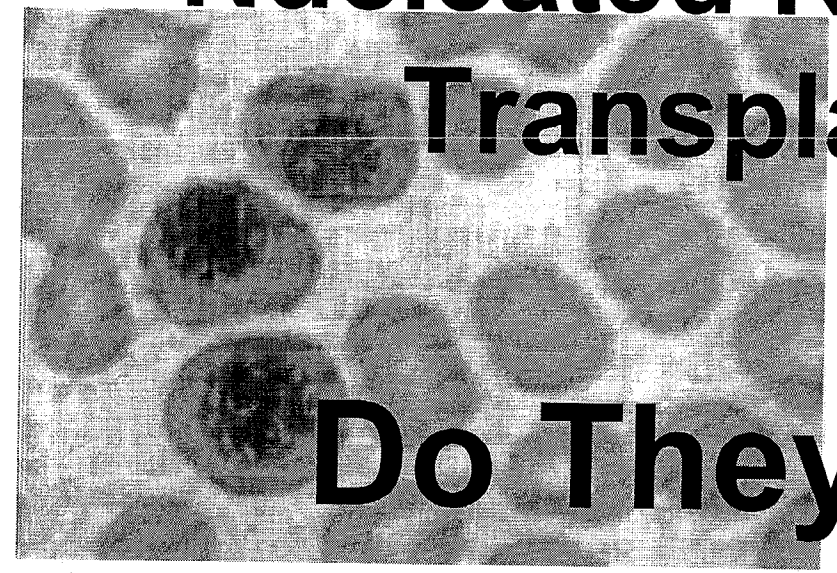
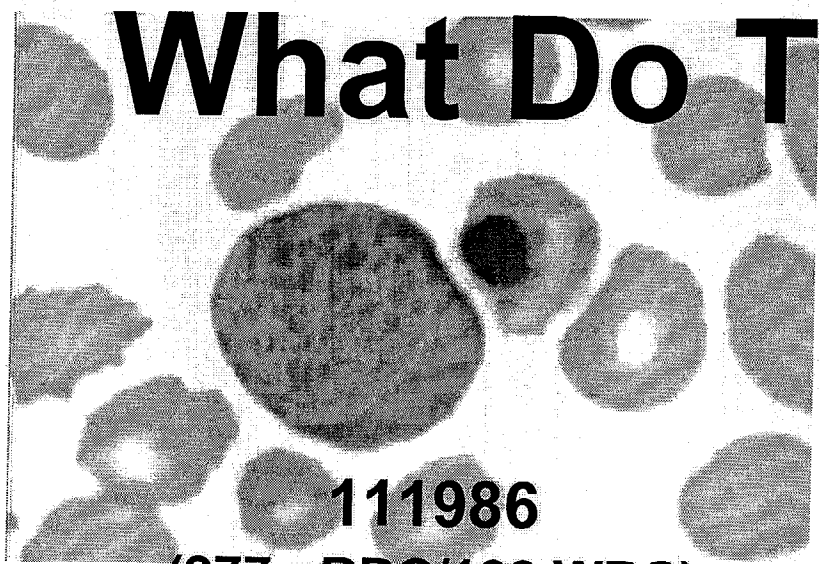
Appendix B

Nucleated RBCs in PCB Transplantation

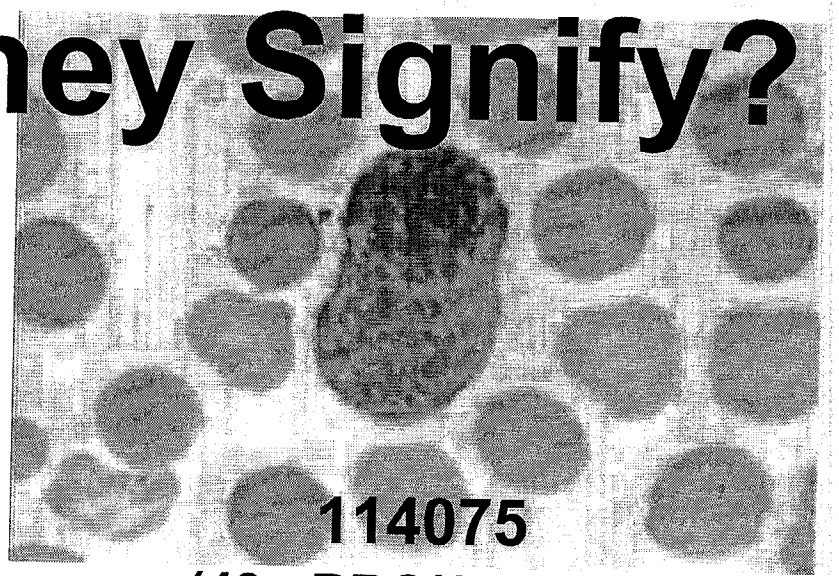
Do They Matter?

What Do They Signify?





111986
(277 nRBC/100 WBC)



114075
(49 nRBC/100 WBC)

Nucleated RBC Study

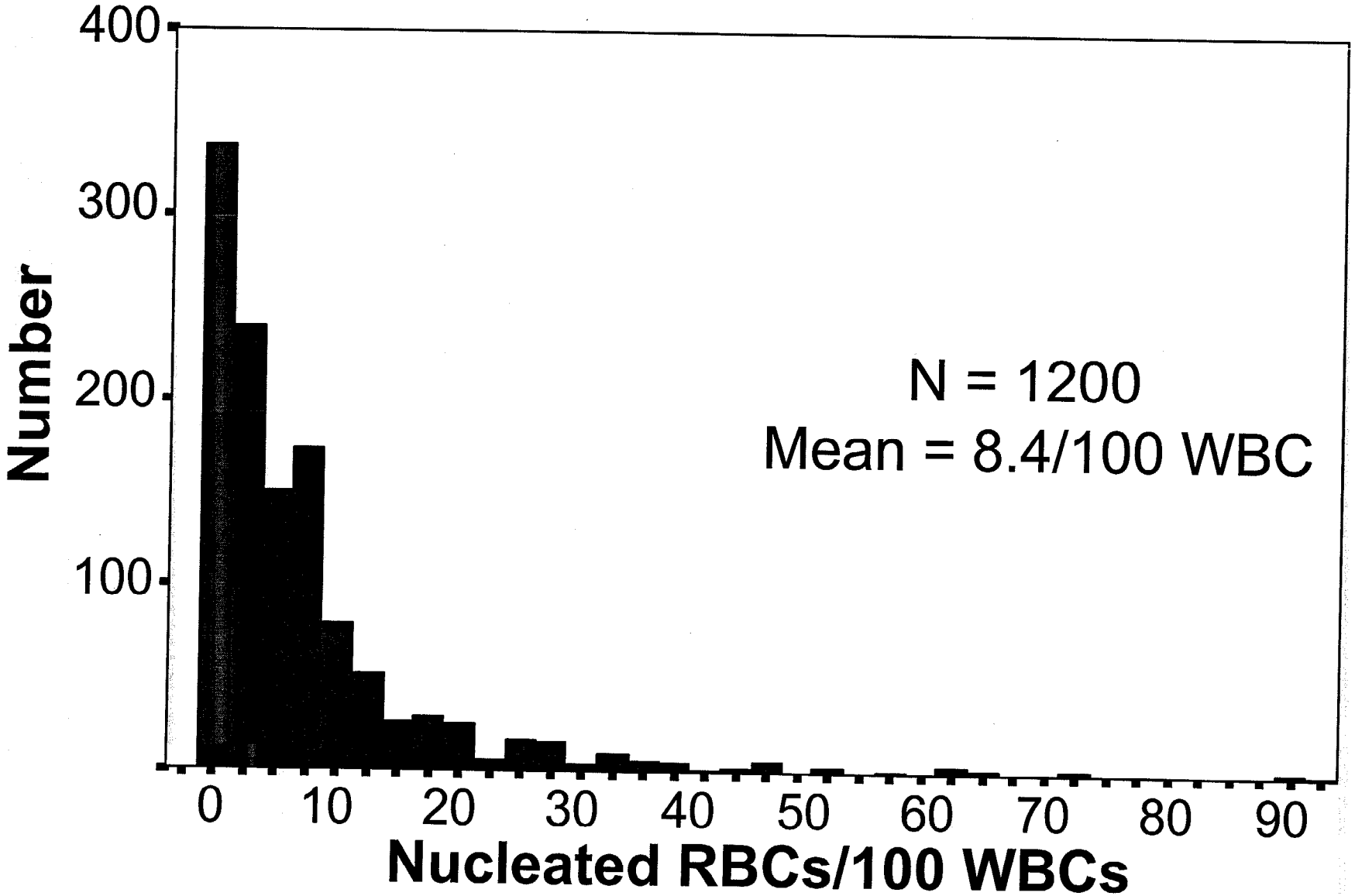
Retrospective Exam of Blood Smears from:

933 PCB Units Transplanted through 12/99

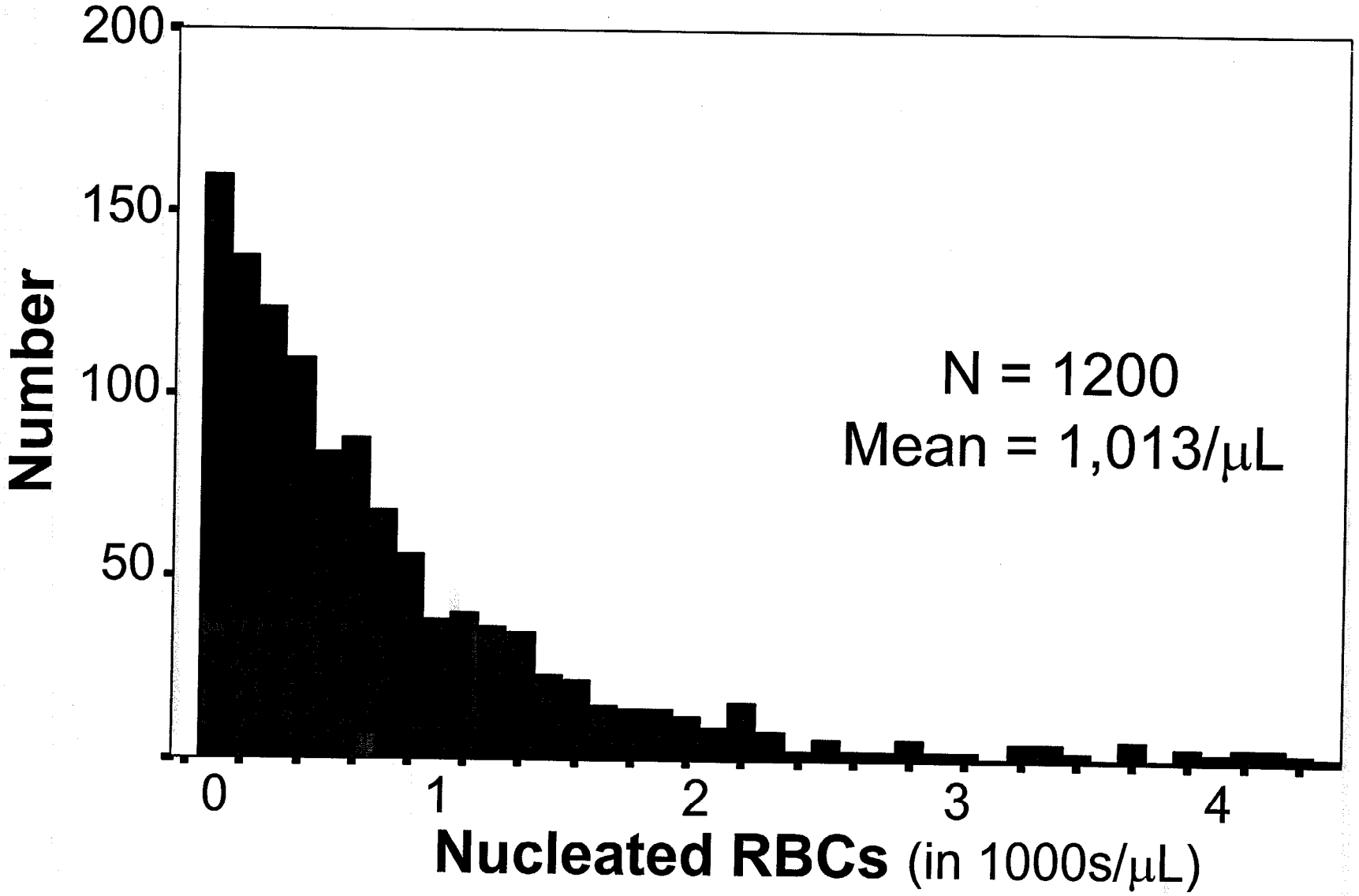
1200 PCB Units Random/Stratified Sample:

- 300 Asian
- 300 African-American
- 300 Hispanic
- 300 Caucasian

Distribution of Nucleated RBCs/100 WBCs



Distribution of Nucleated RBC Number

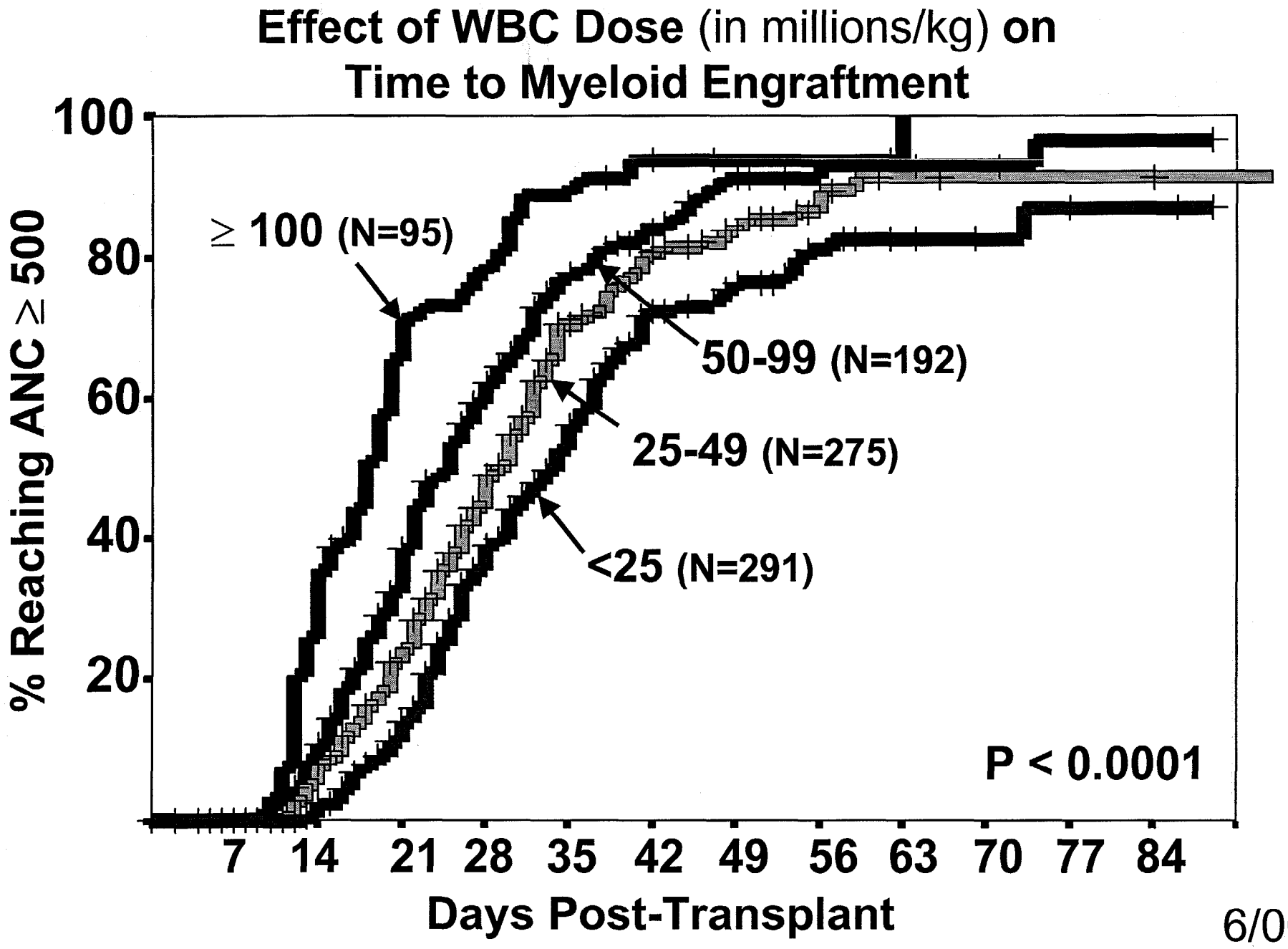


Issues

Artifact in TNC Dose?

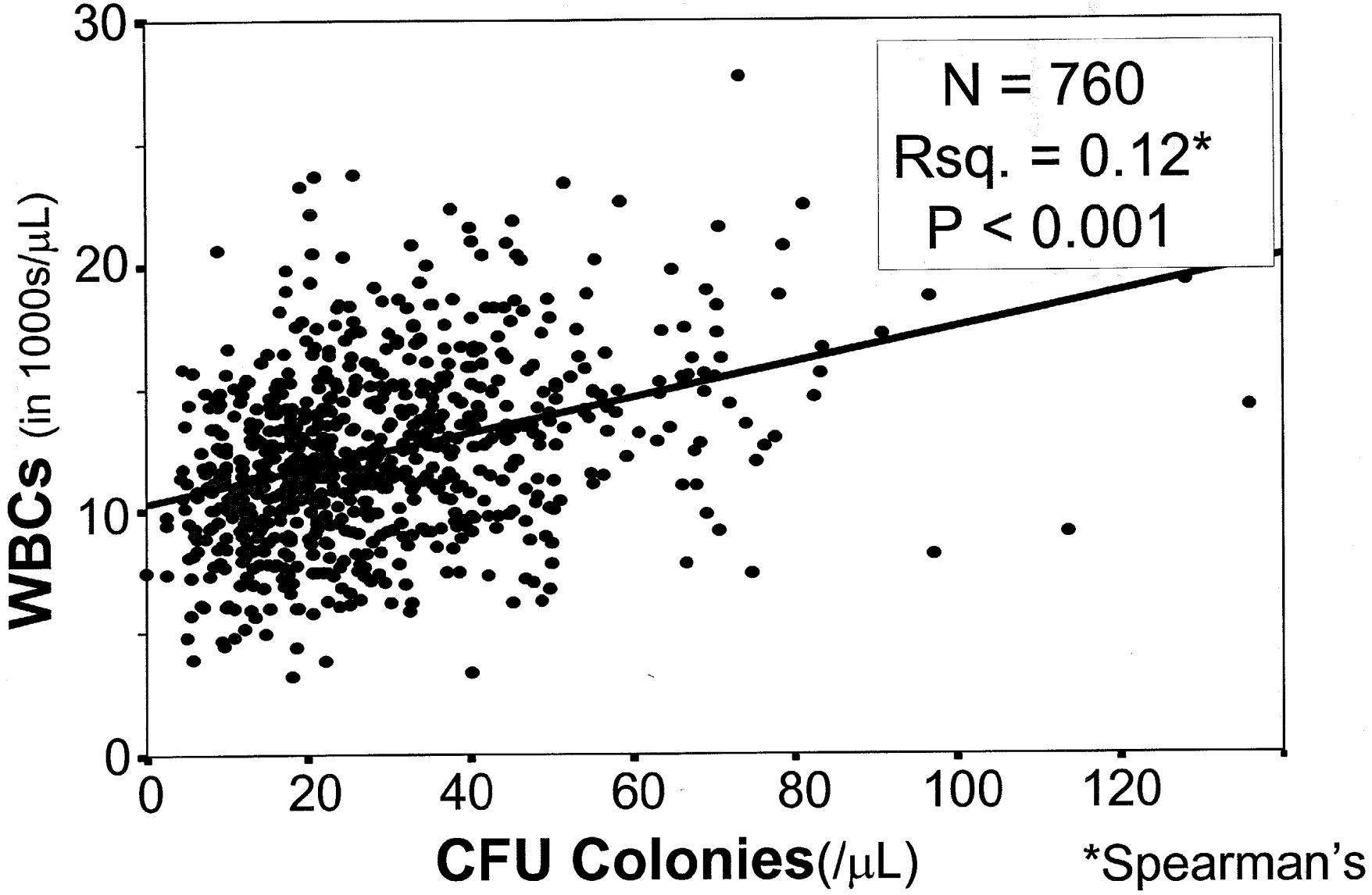
Disease Flag?

Biological Implications?


Effect of WBC Dose (in millions/kg) on
Time to Myeloid Engraftment
% Reaching ANC ≥ 500
100
80
60
40
20
≥ 100 (N=95)
50-99 (N=192)
25-49 (N=275)
<25 (N=291)
P < 0.0001
7
14
21
28
35
42
49
56
63
70
77
84
Days Post-Transplant

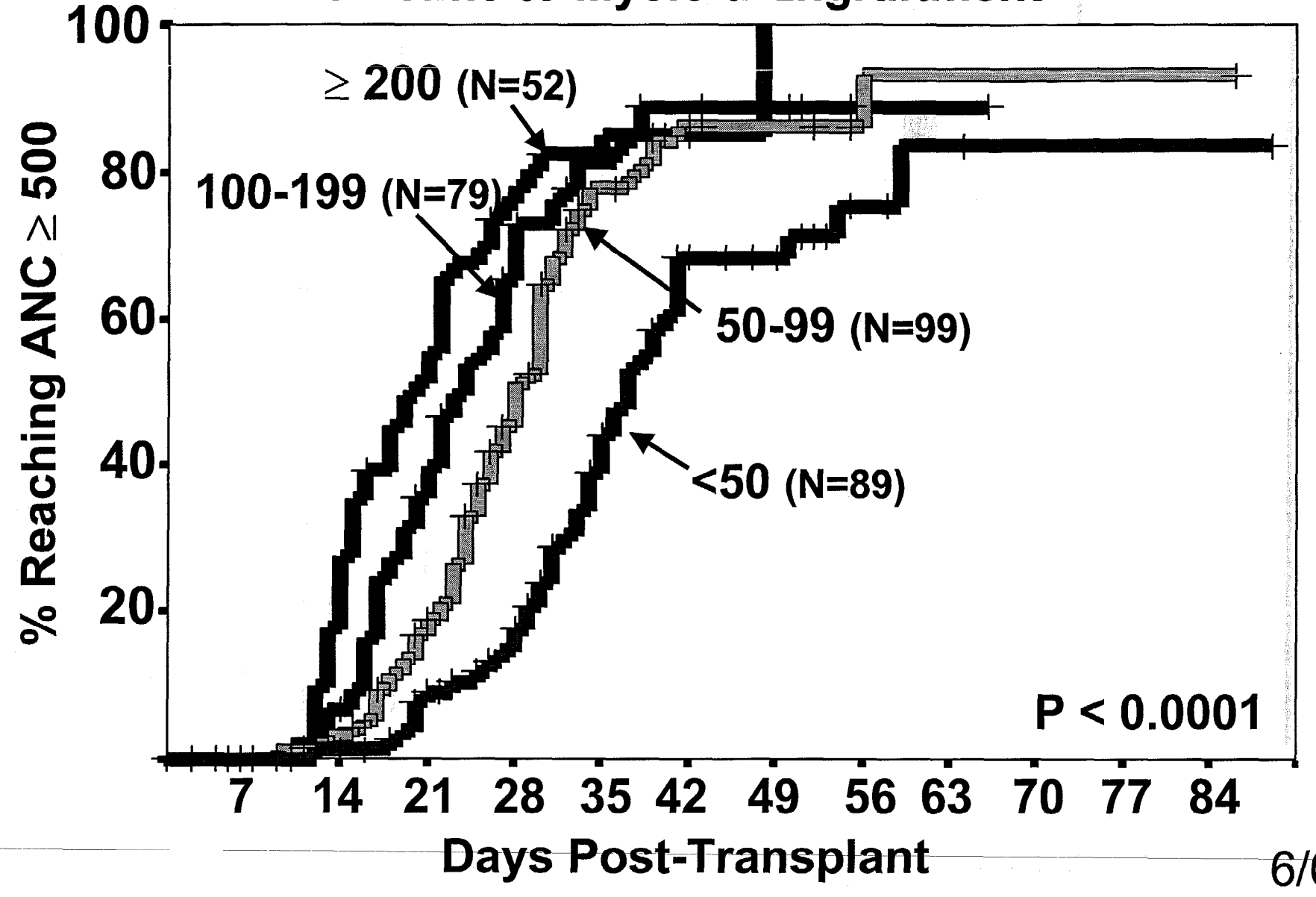
6/00

Correlation of WBC with CFU Colonies



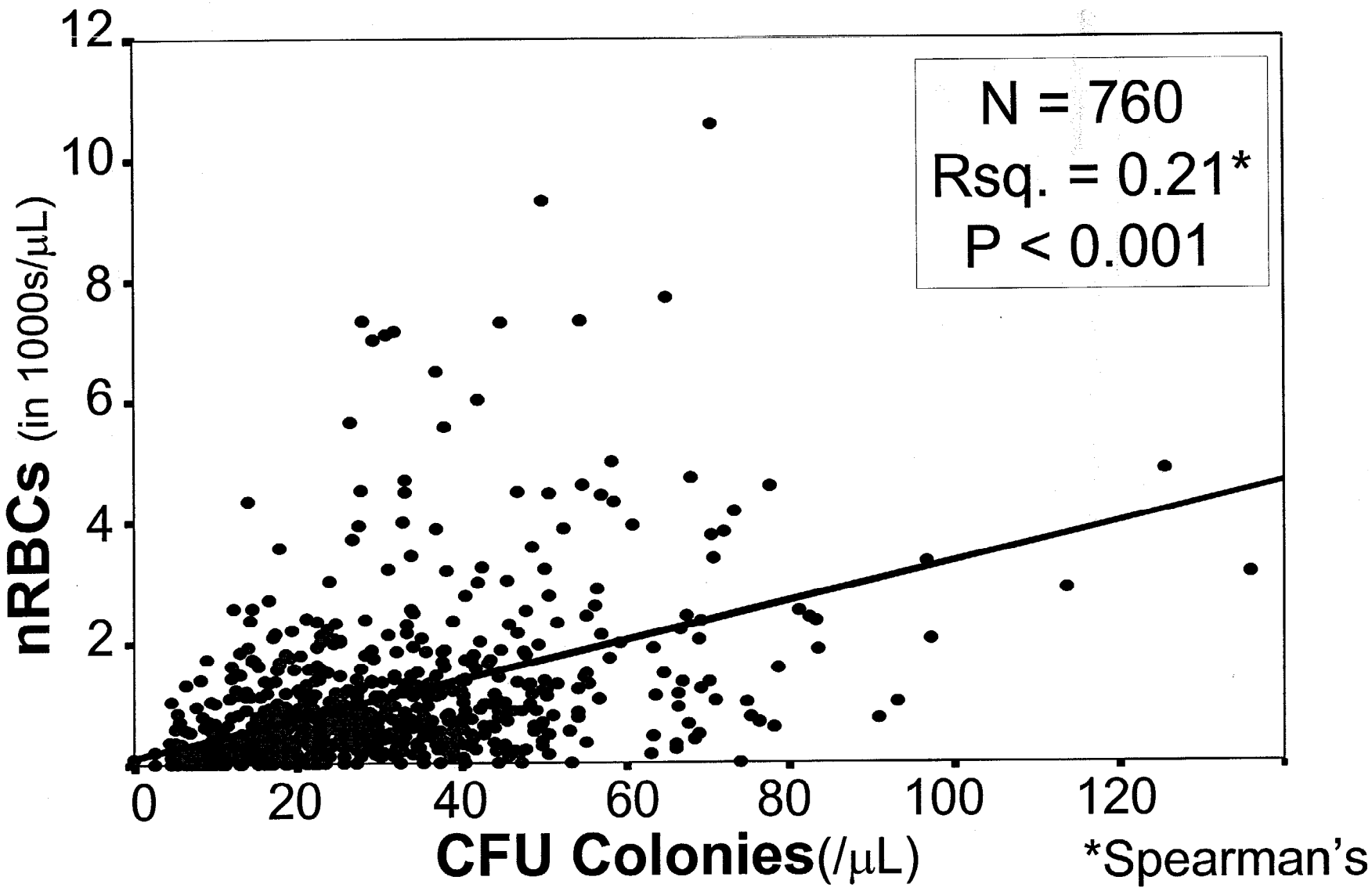
*Spearman's

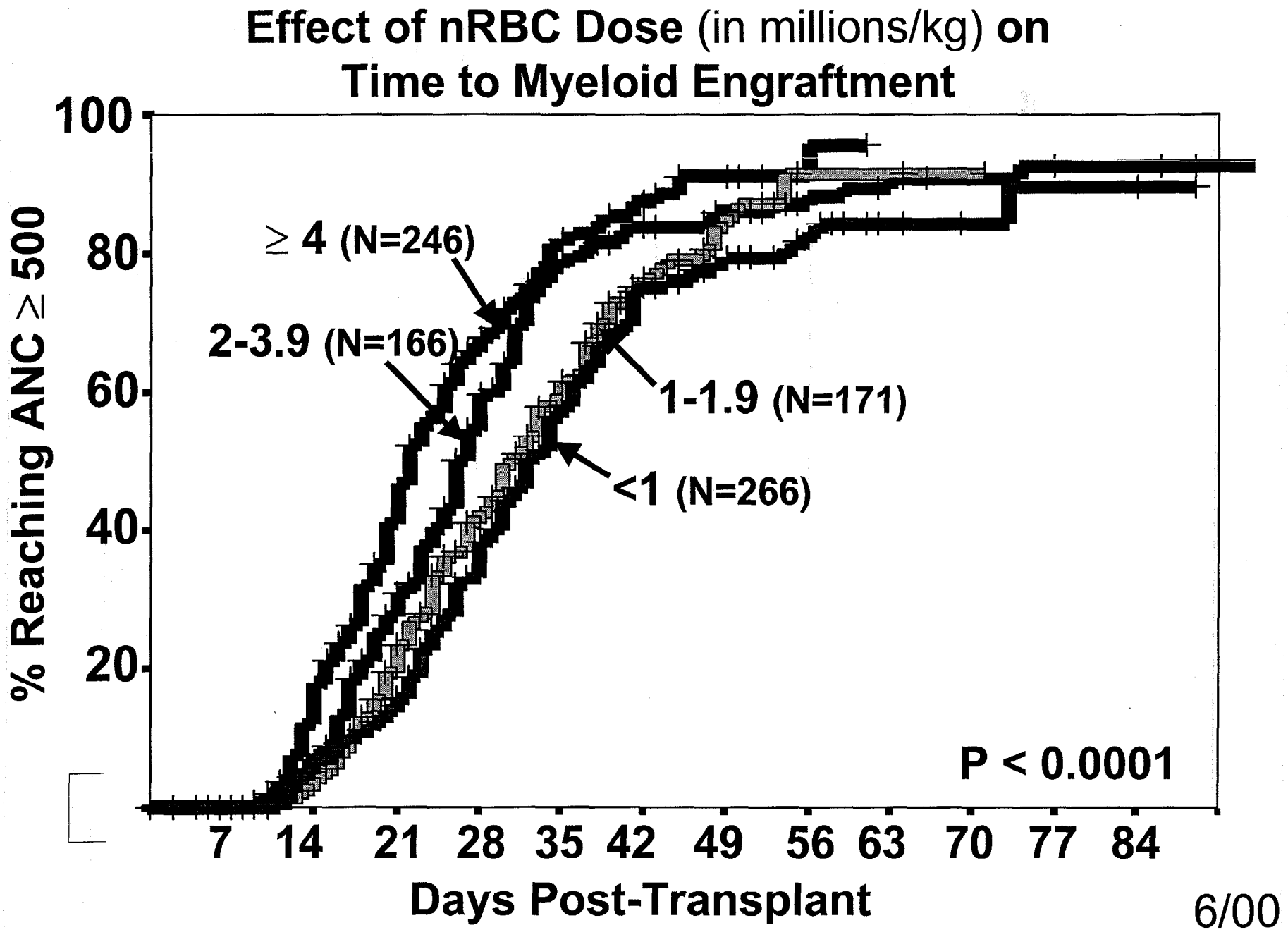
Effect of CFU Colony Dose (in thousands/kg) on Time to Myeloid Engraftment



6/00

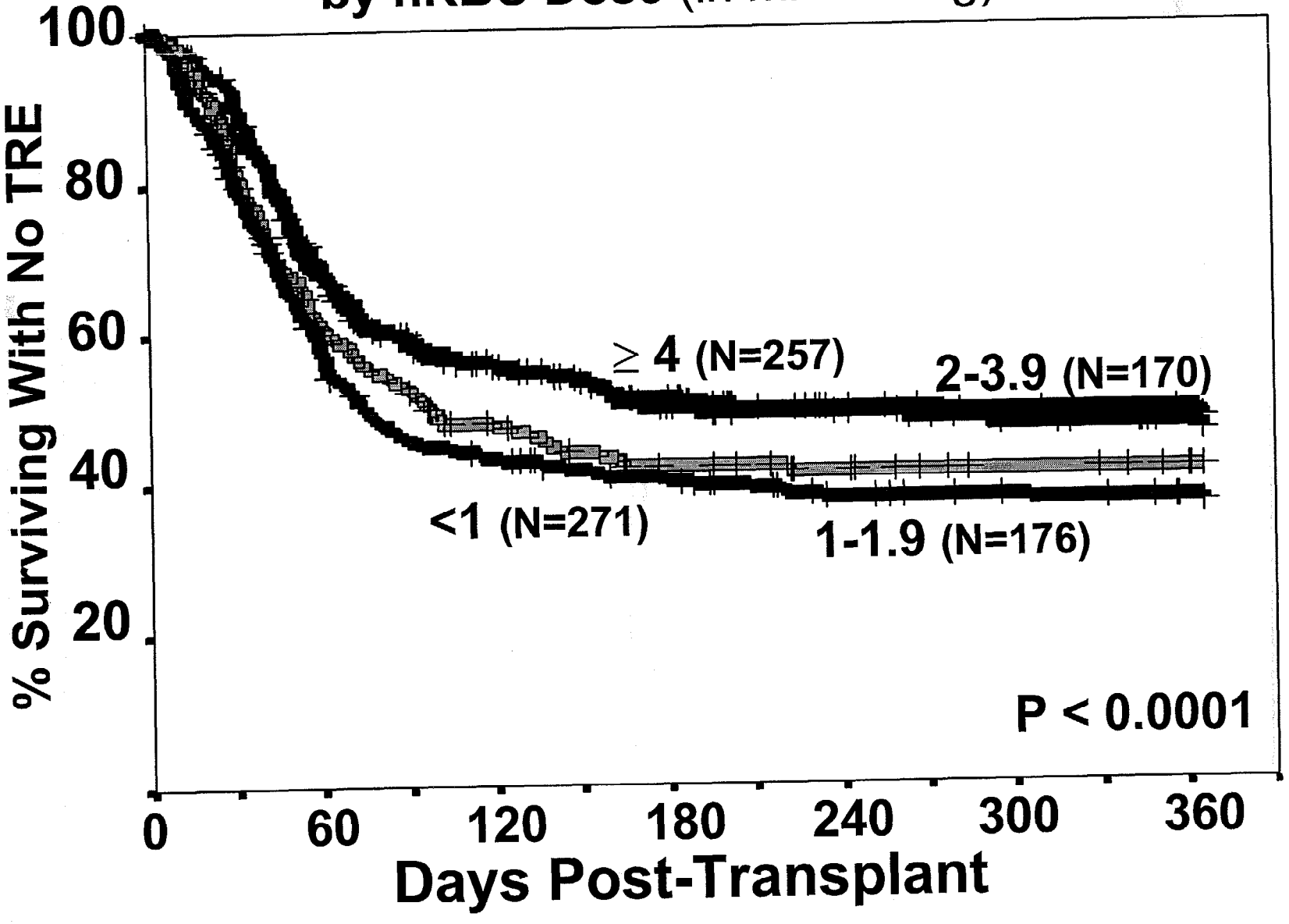
Correlation of nRBC with CFU Colonies




Effect of nRBC Dose (in millions/kg) on
Time to Myeloid Engraftment
% Reaching ANC ≥ 500
100
80
60
40
20
≥ 4 (N=246)
2-3.9 (N=166)
1-1.9 (N=171)
<1 (N=266)
P < 0.0001
7
14
21
28
35
42
49
56
63
70
77
84
Days Post-Transplant

6/00

Survival With No Transplant-Related Event (TRE) by nRBC Dose (in millions/kg)



6/00

Relative Risks for Faster Engraftment

Variable	Relative Risks*			
	Univariate		Multivariate	
	RR	P value	RR	P value
WBC:≥100	1.2	<.001	.4	.3
50-99	.6	<.001	-.3	.4
25-49	.3	.025	.2	.4
CFU:≥200	1.4	<.001	1.3	<.001
100-199	1.2	<.001	1.3	<.001
50-99	.8	<.001	.8	<.001
nRBC:≥2	.5	<.001	.1	.7

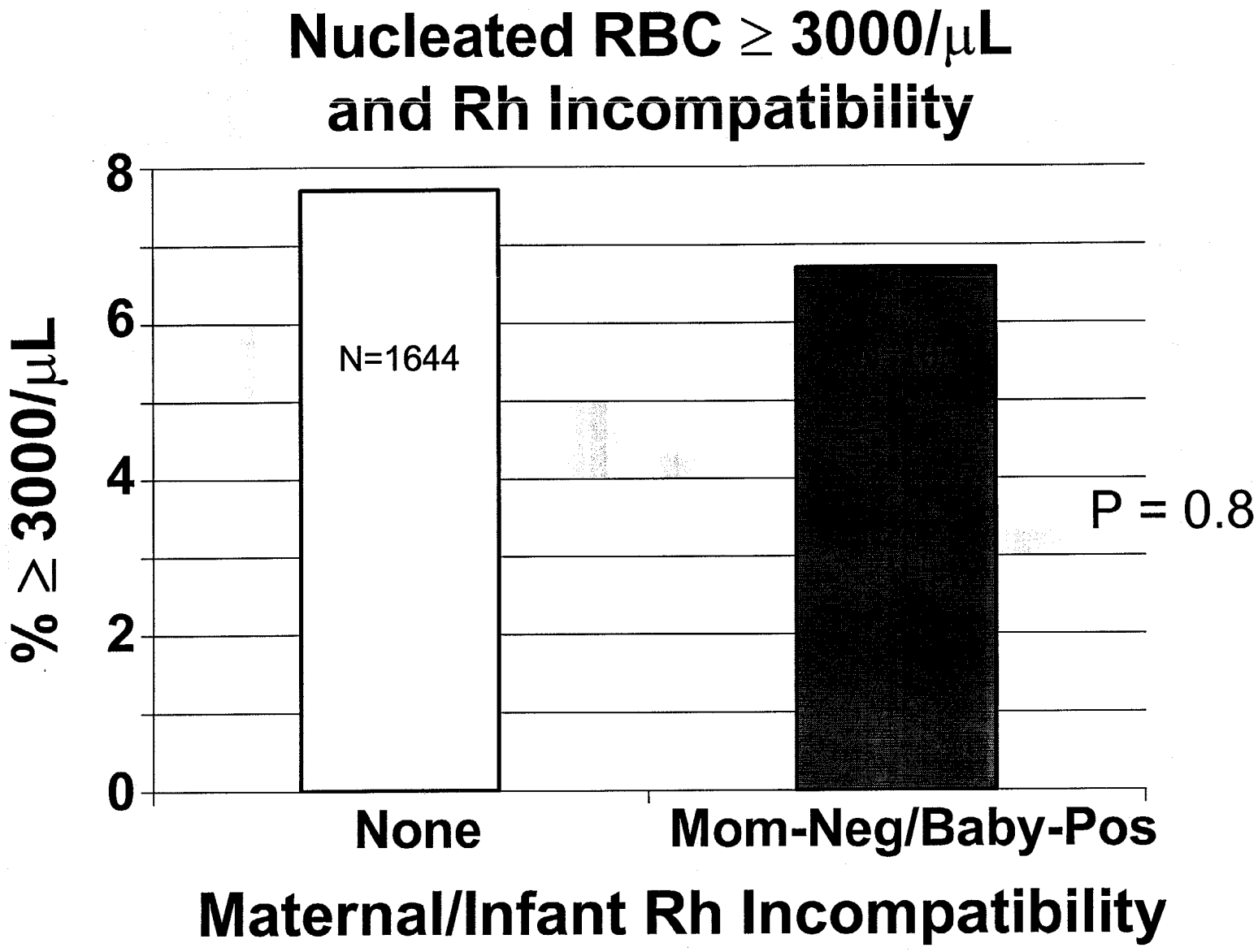
*Cox Regression. Reference = Lowest Doses/Kg

Issues

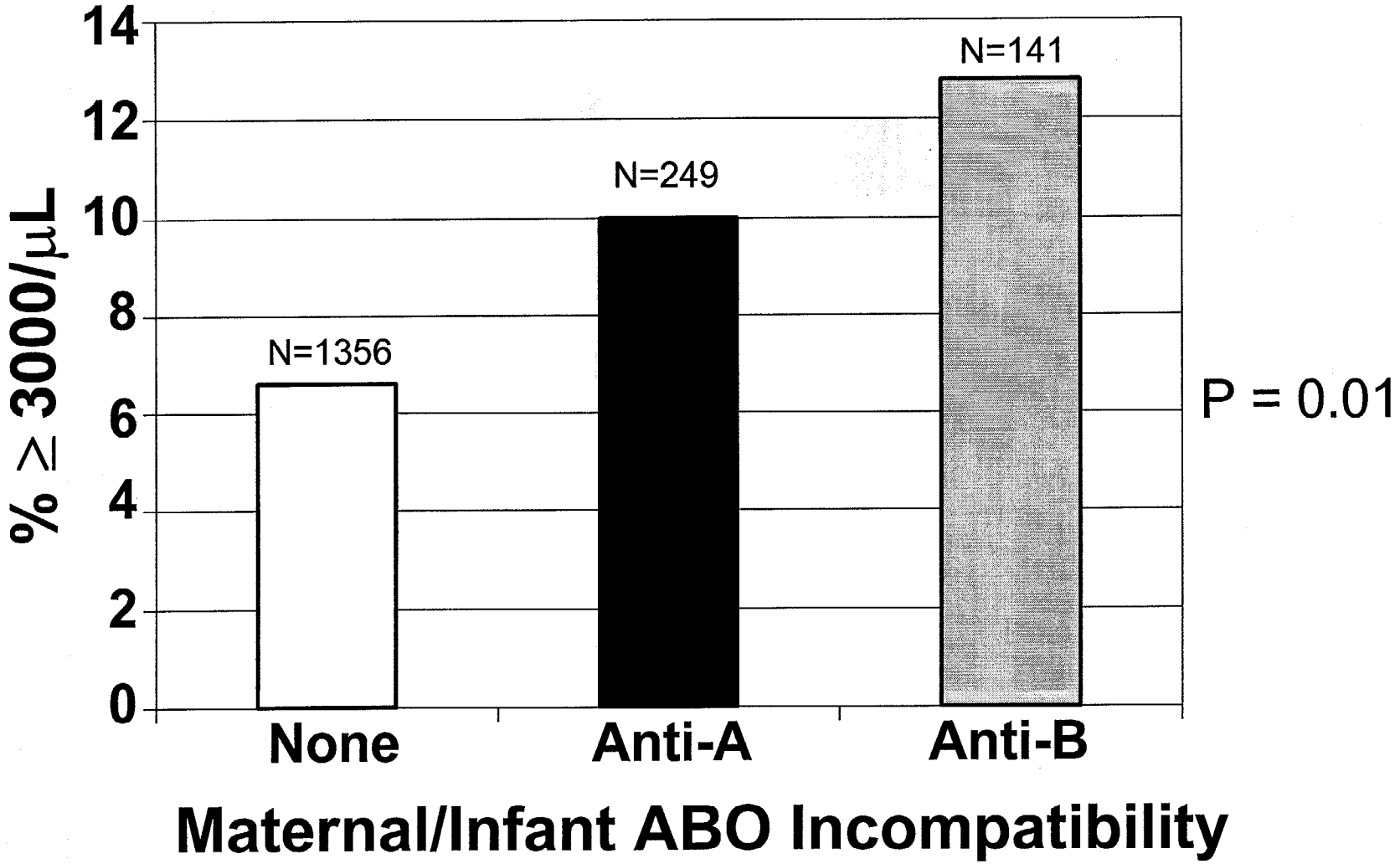
Artifact for TNC Dose?

Disease Flag?

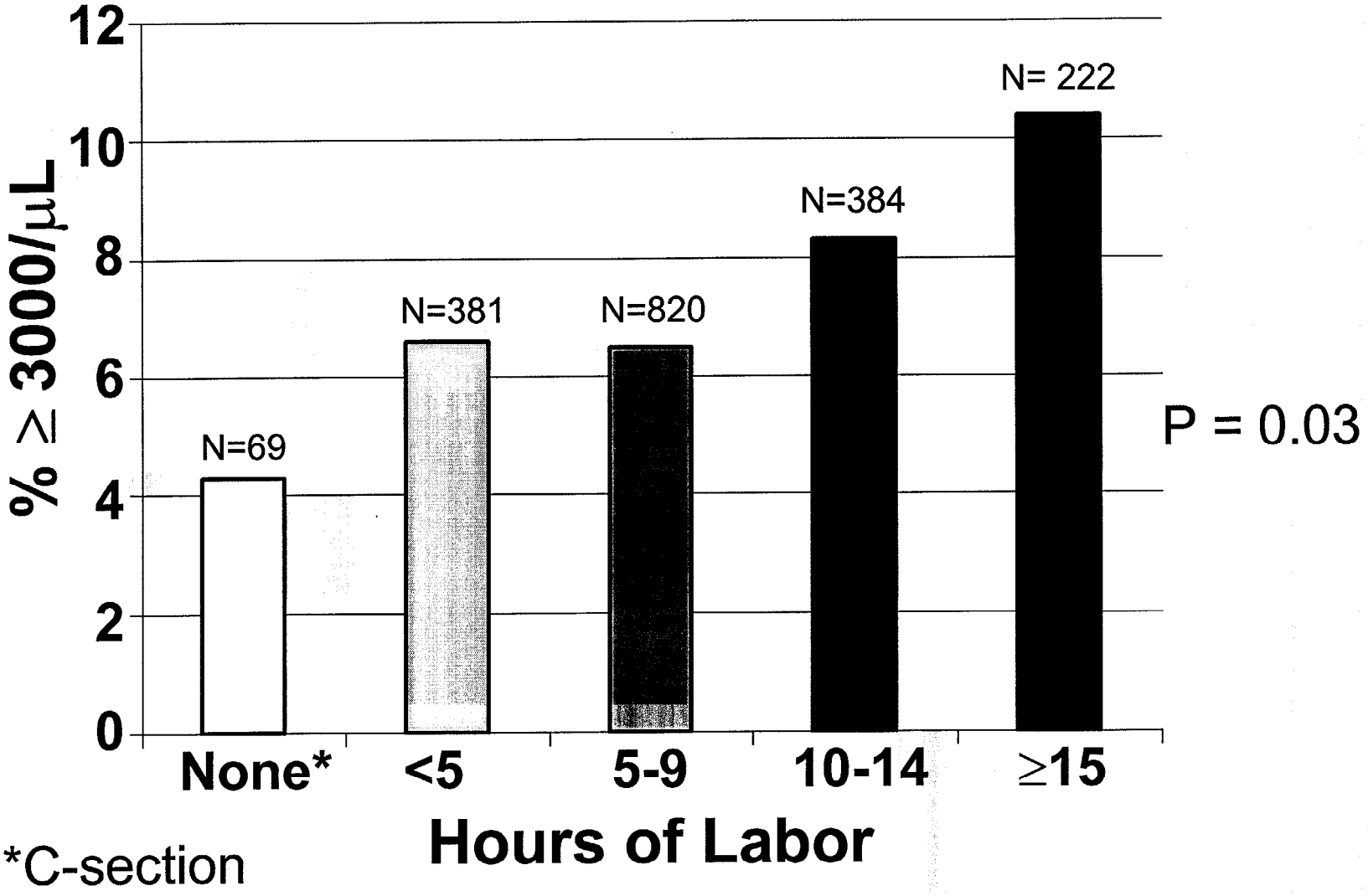
Biological Implications?


Nucleated RBC ≥ 3000/μL
and Rh Incompatibility
% ≥ 3000/μL
8
6
4
2
0
N=1644
P = 0.8
None
Mom-Neg/Baby-Pos
Maternal/Infant Rh Incompatibility

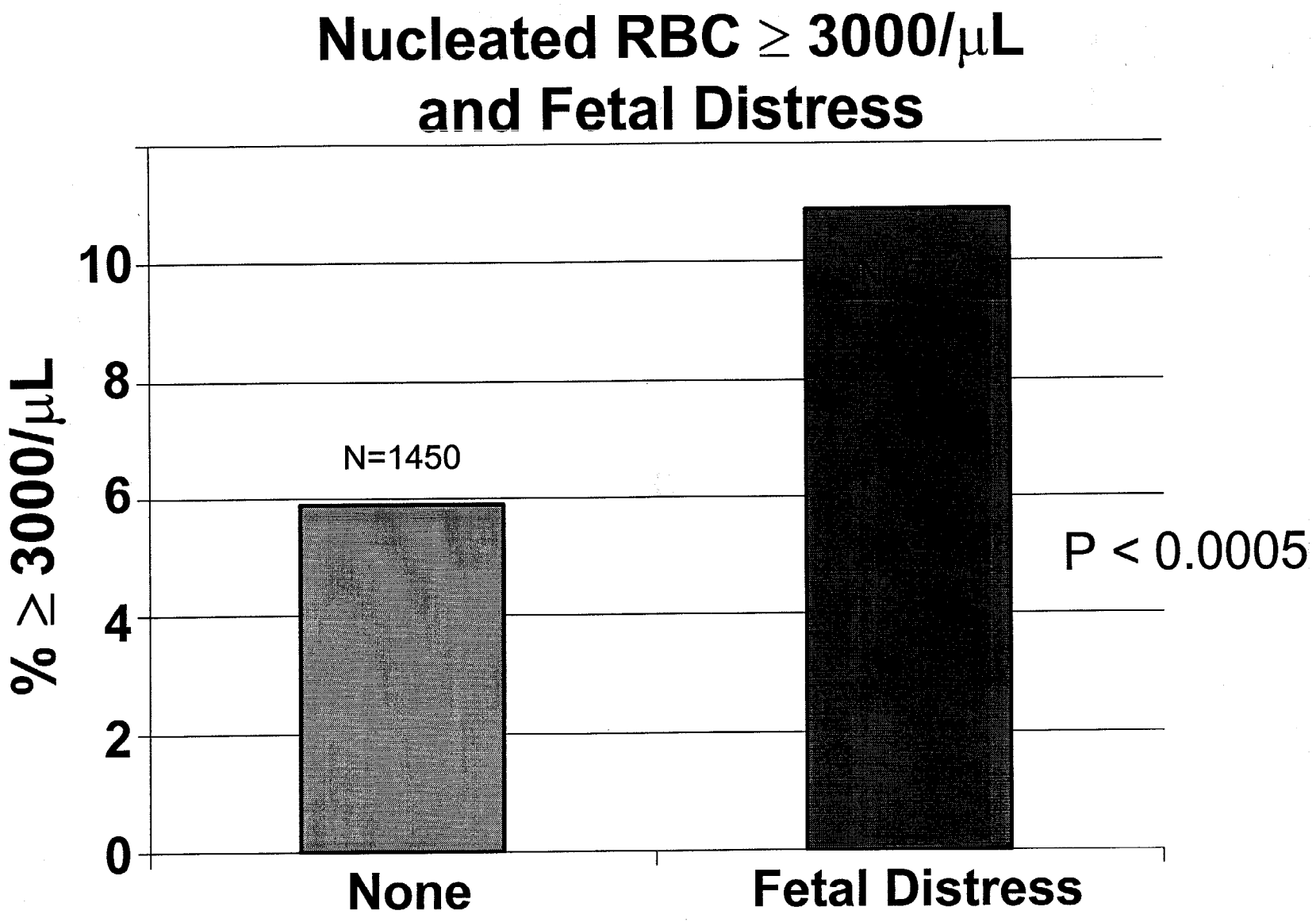
Nucleated RBC ≥ 3000/μL and ABO Incompatibility



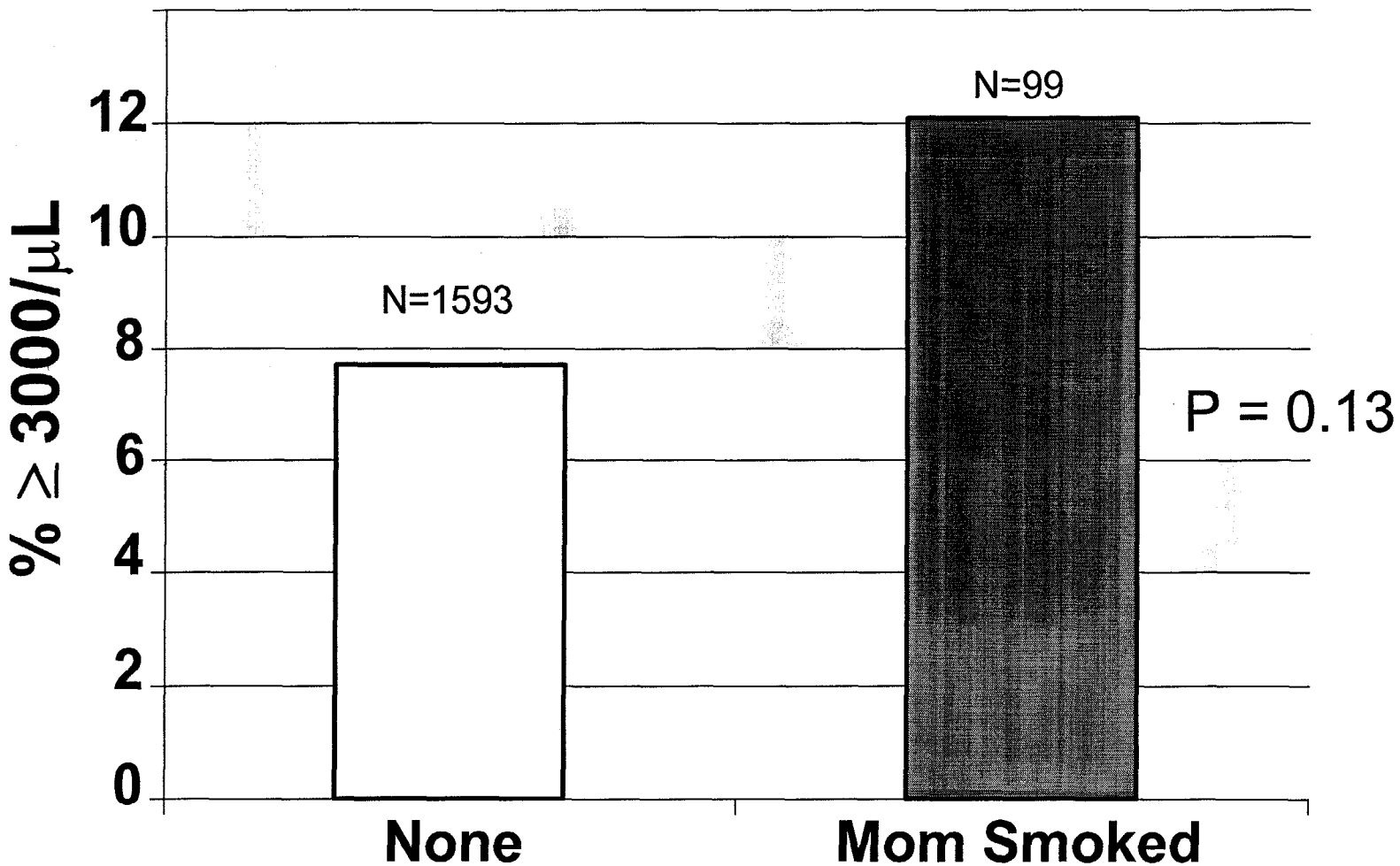
Nulceated RBC ≥ 3000/μL and Length of Labor



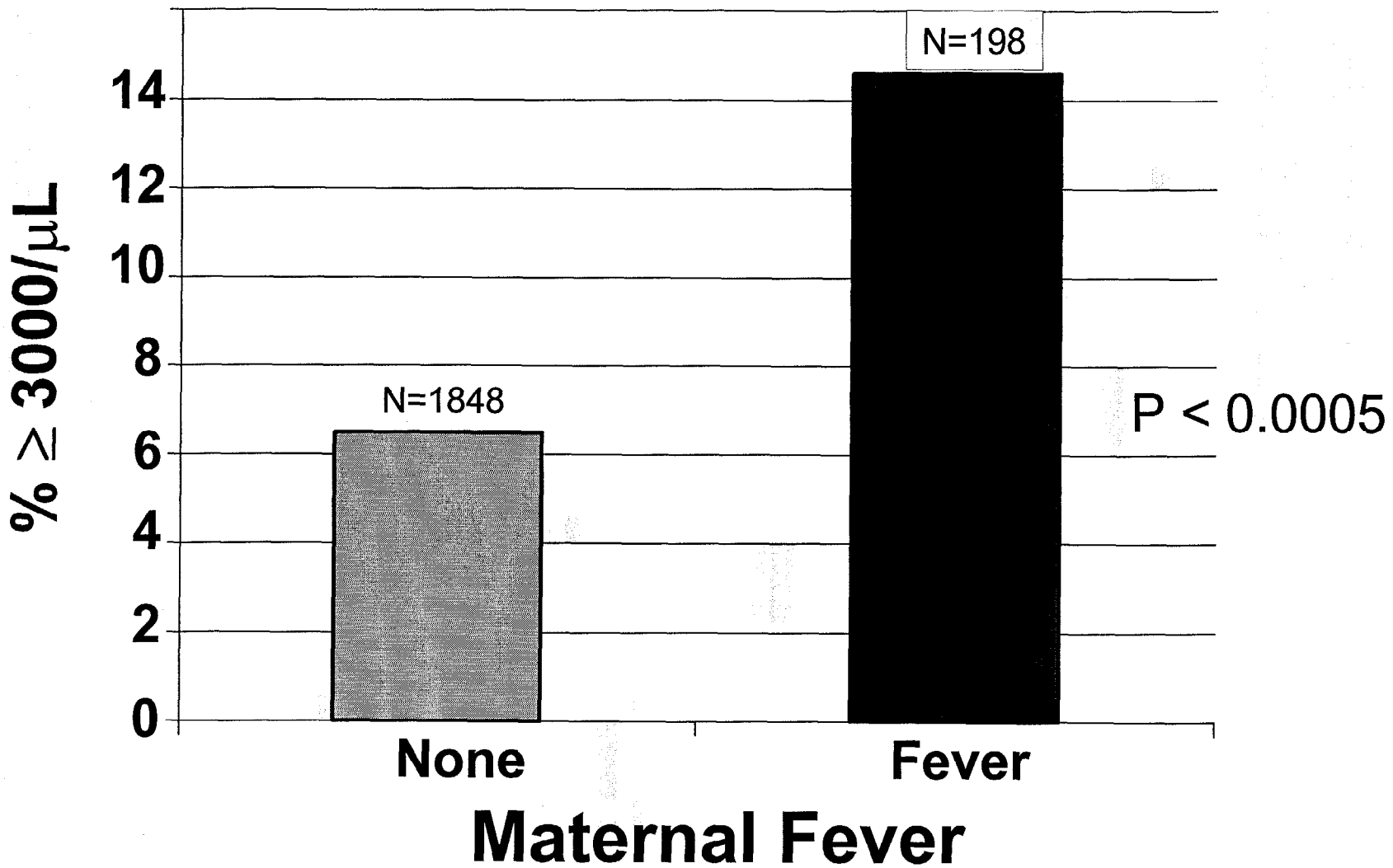
*C-section


Nucleated RBC ≥ 3000/μL
and Fetal Distress
% ≥ 3000/μL
10
8
6
4
2
0
N=1450
P < 0.0005
None
Fetal Distress

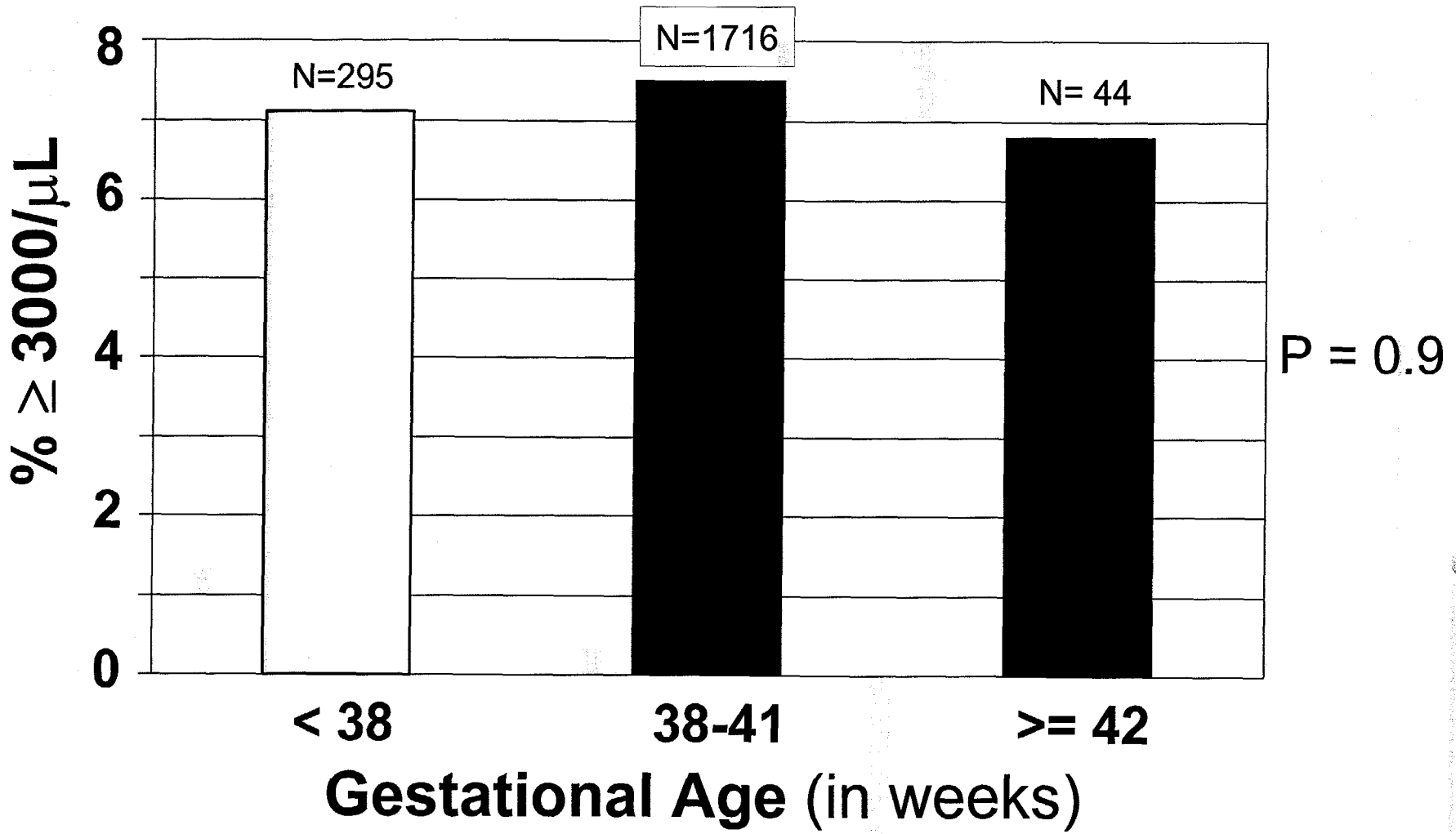
Nucleated RBC ≥ 3000/μL and Maternal Smoking During Pregnancy



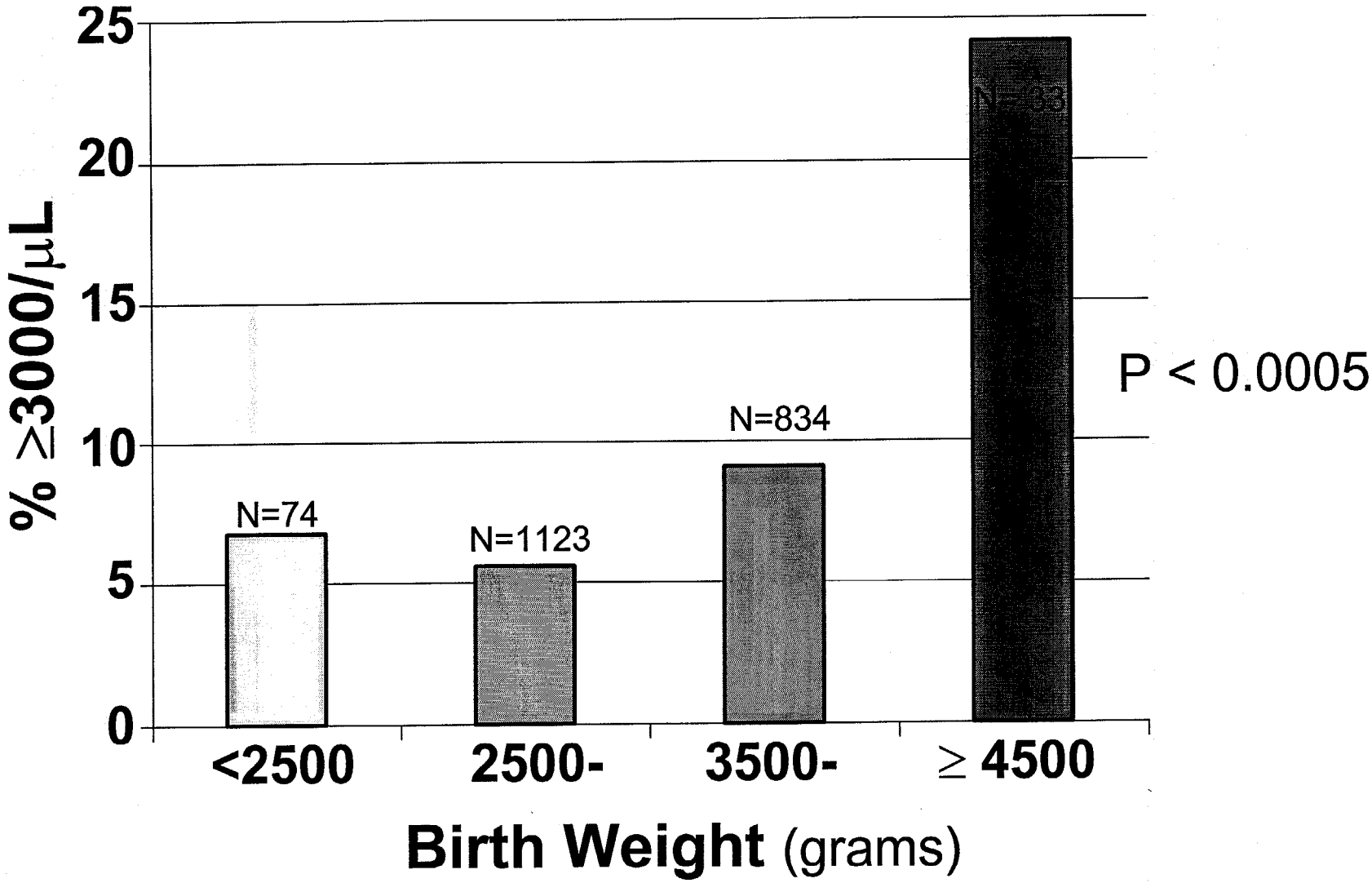
Nulceated RBC≥3000/μL and Fever during Labor and Delivery



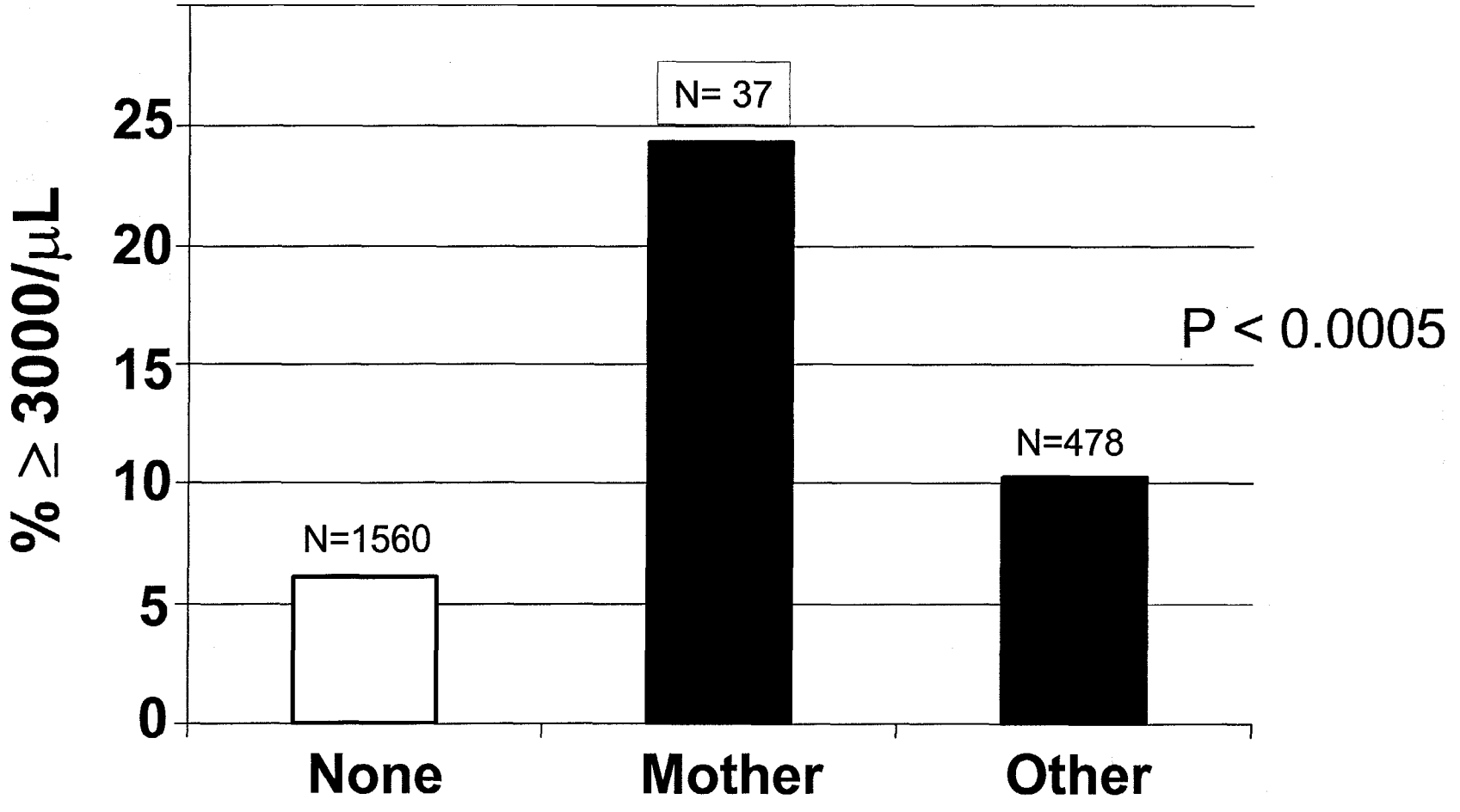
Nucleated RBC ≥ 3000/μL and Gestational Age at Birth



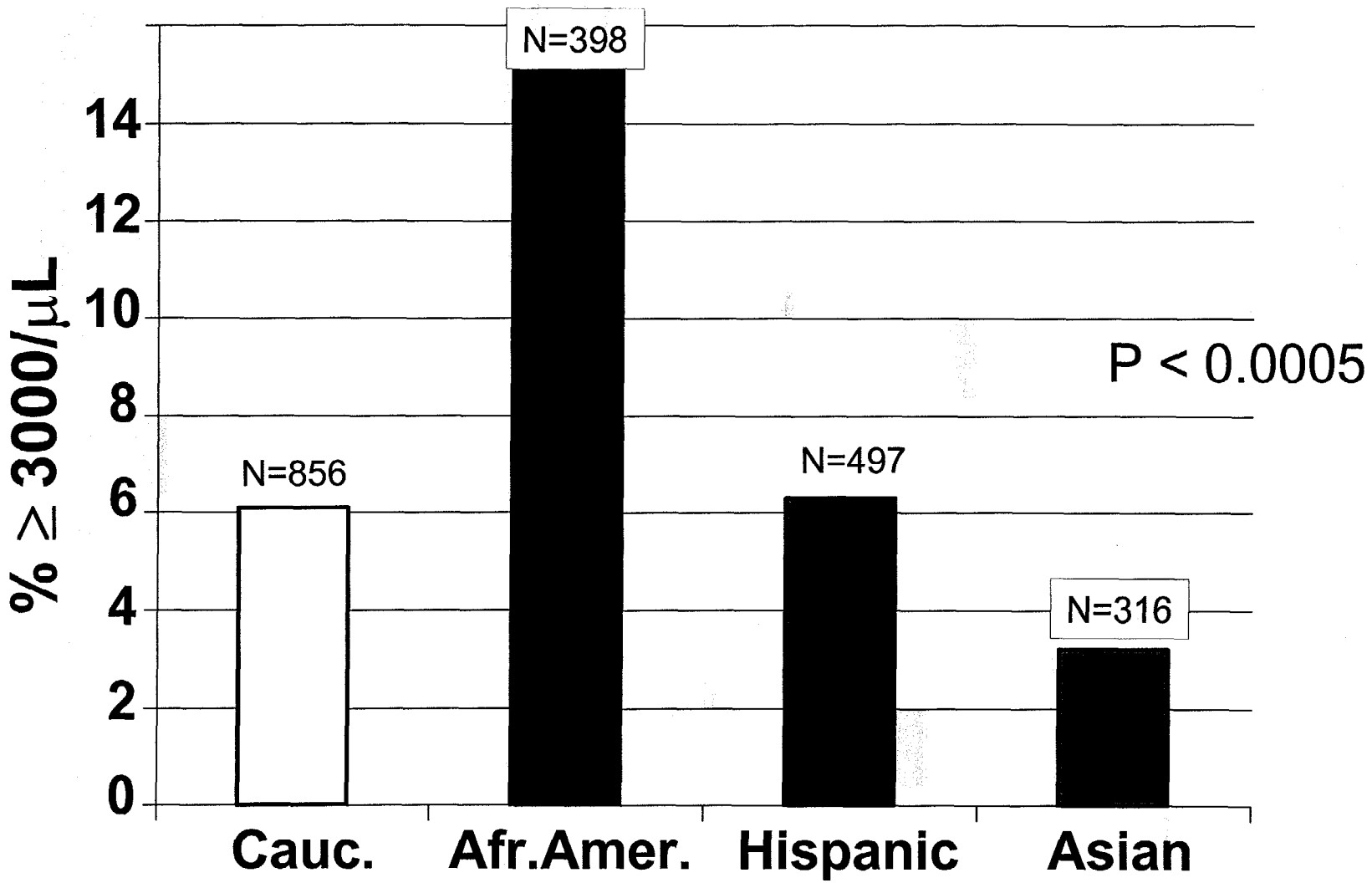
Nulceated RBC ≥ 3000/μL and Birth Weight



Nucleated RBC ≥ 3000/μL and Family History of Diabetes Mellitus



Nucleated RBC ≥ 3000/μL and Mother's Ethnicity



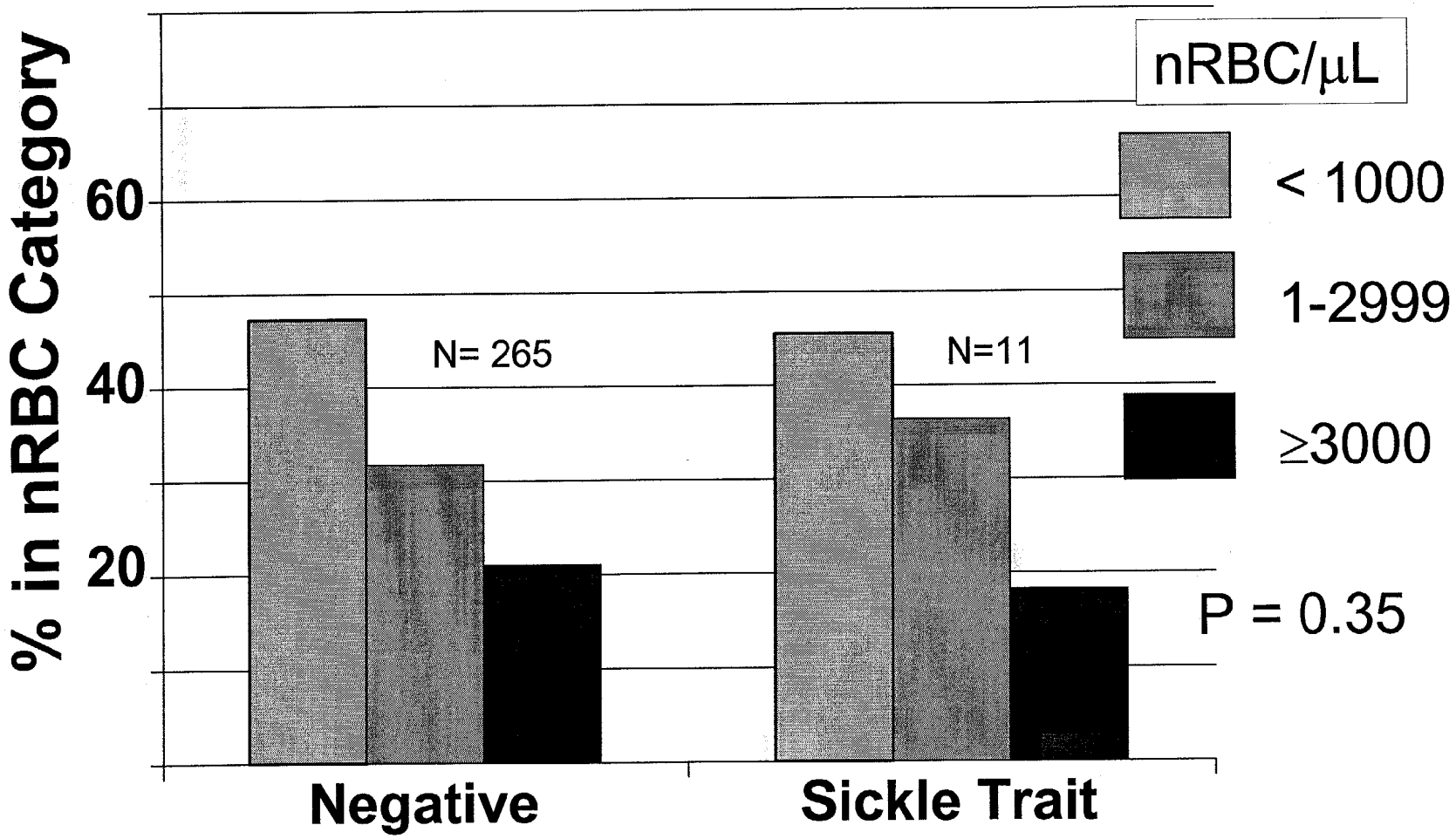
Multivariate Analysis of Factors Associated with Nucleated RBC Count

Variable	Odds Ratio*	P value
Ethnicity: Asian	-2.0 (-5 – 0.8)	0.17
African-Amer.	7.3 (5 - 10)	<0.0005
Hispanic	0.5 (-2 – 3)	0.6
ABO Incomp.: A	2.1 (-.4 – 5)	0.099
B	7.5 (4 -11)	<0.0005
Fetal Distress	3.3 (2 - 5)	<0.0005
Maternal Fever	2.8 (-.1 - 6)	0.07
Birth Weight (in grams)	0.04 (.02 -.05)	<0.0005

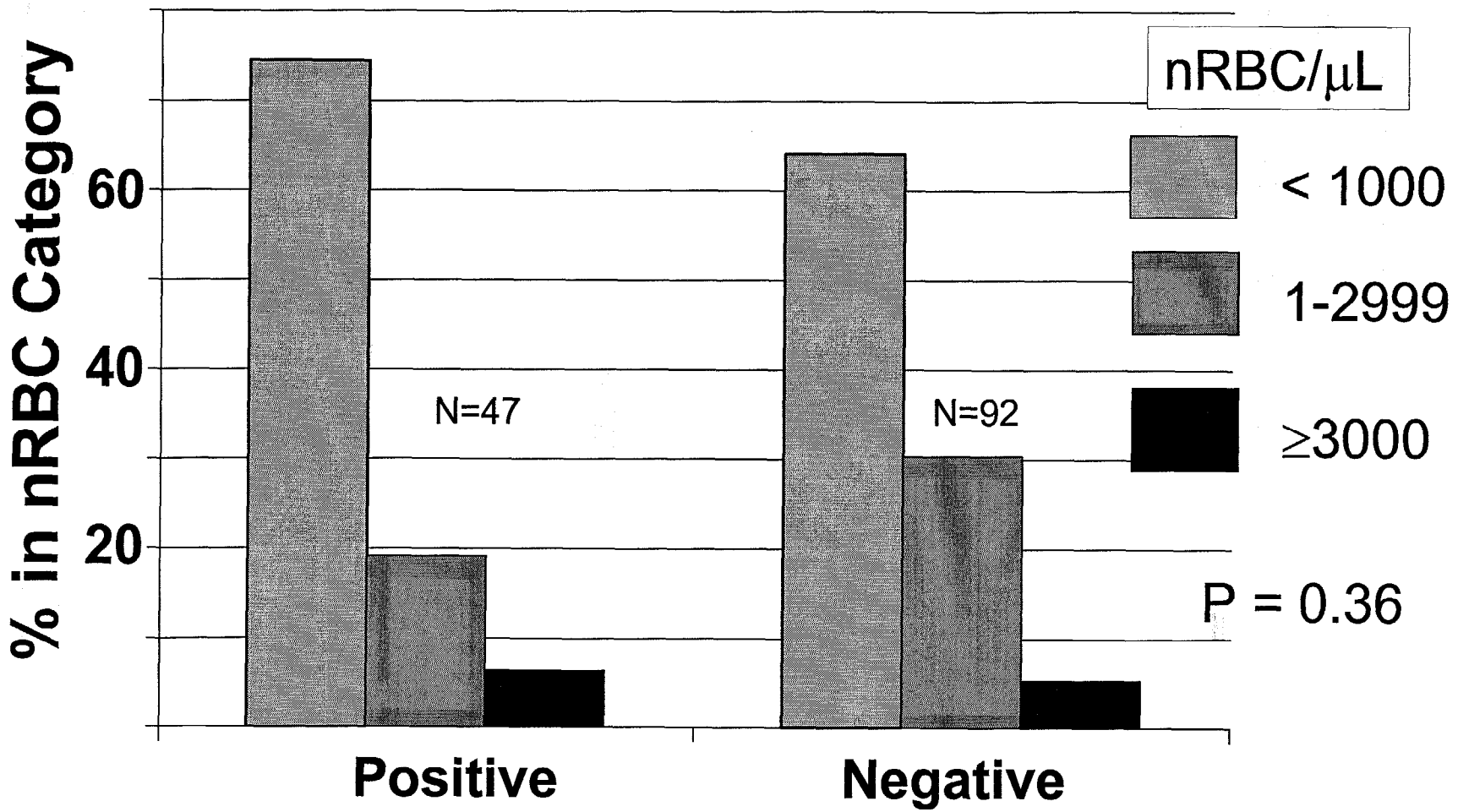
Diabetes, Apgar, Length of Labor not independently predictive of nRBC count.

* By Linear Regression, ( ) = 95%ile.
Reference = Others in the Group

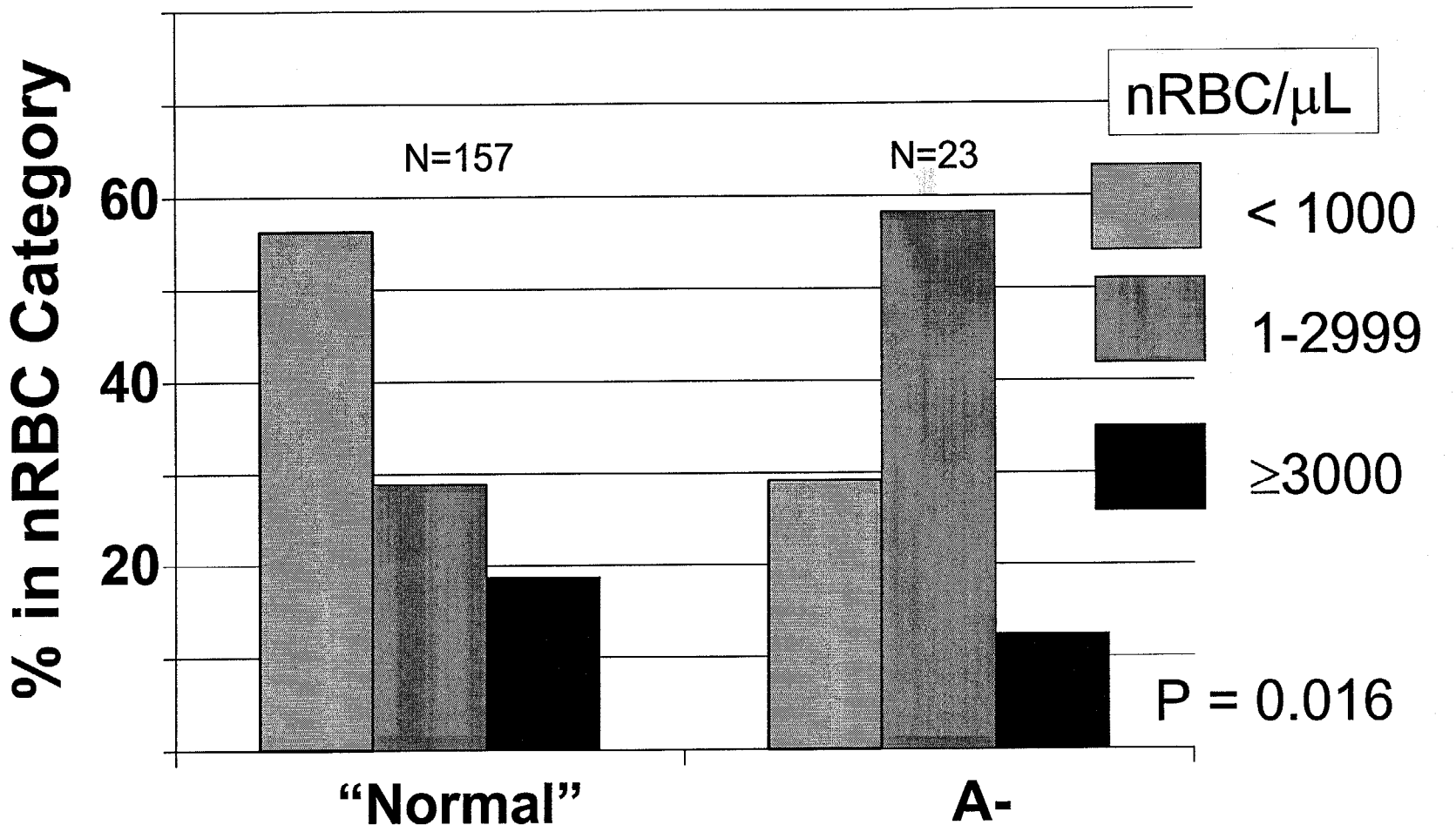
Nucleated RBC/μL and Sickle Hemoglobin in African-Americans



Nucleated RBC/μL and Duffy Blood Group in African-Americans

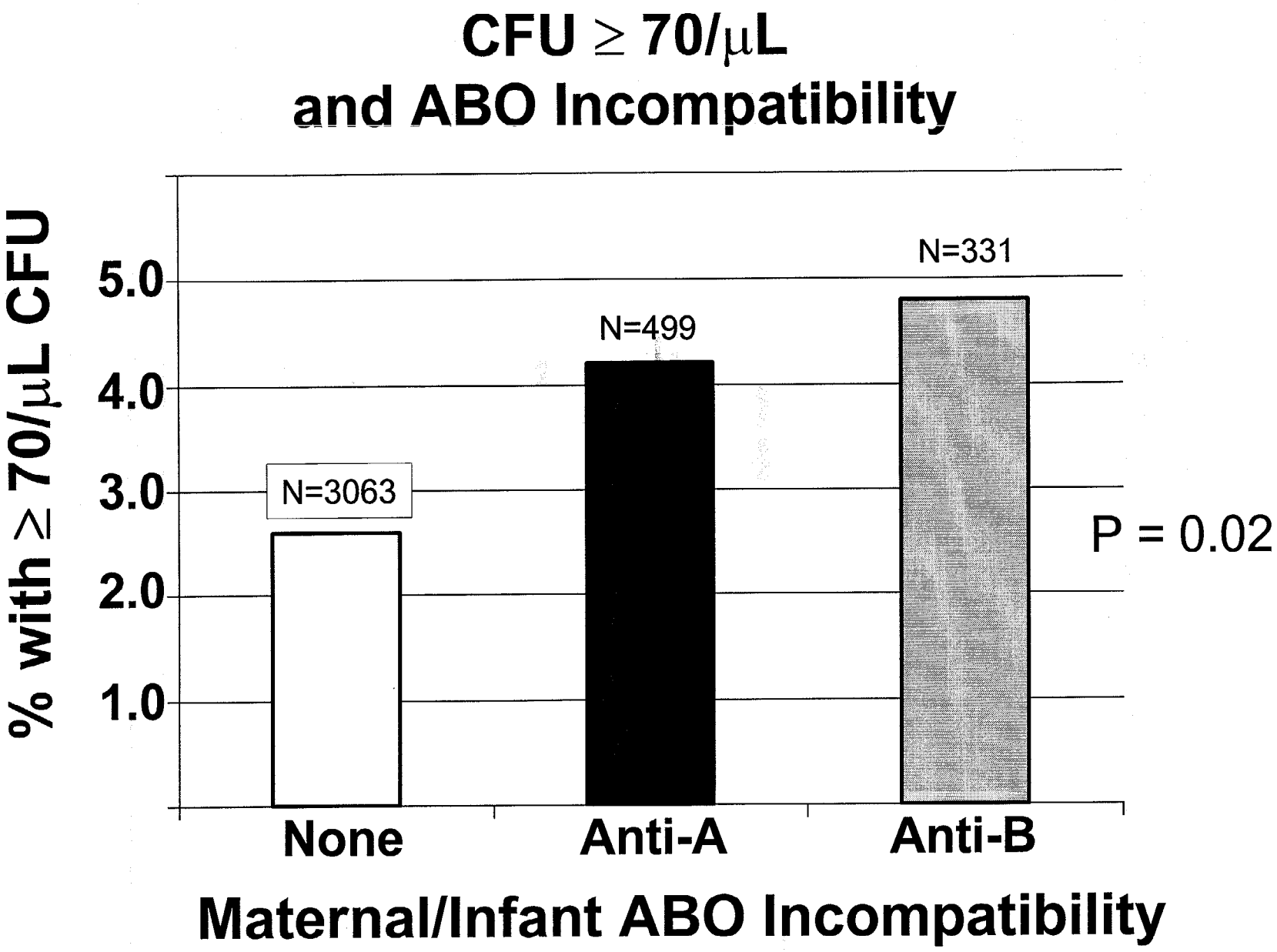


Nucleated RBC/μL and G6PD A- Variant* in African-Amer. Males

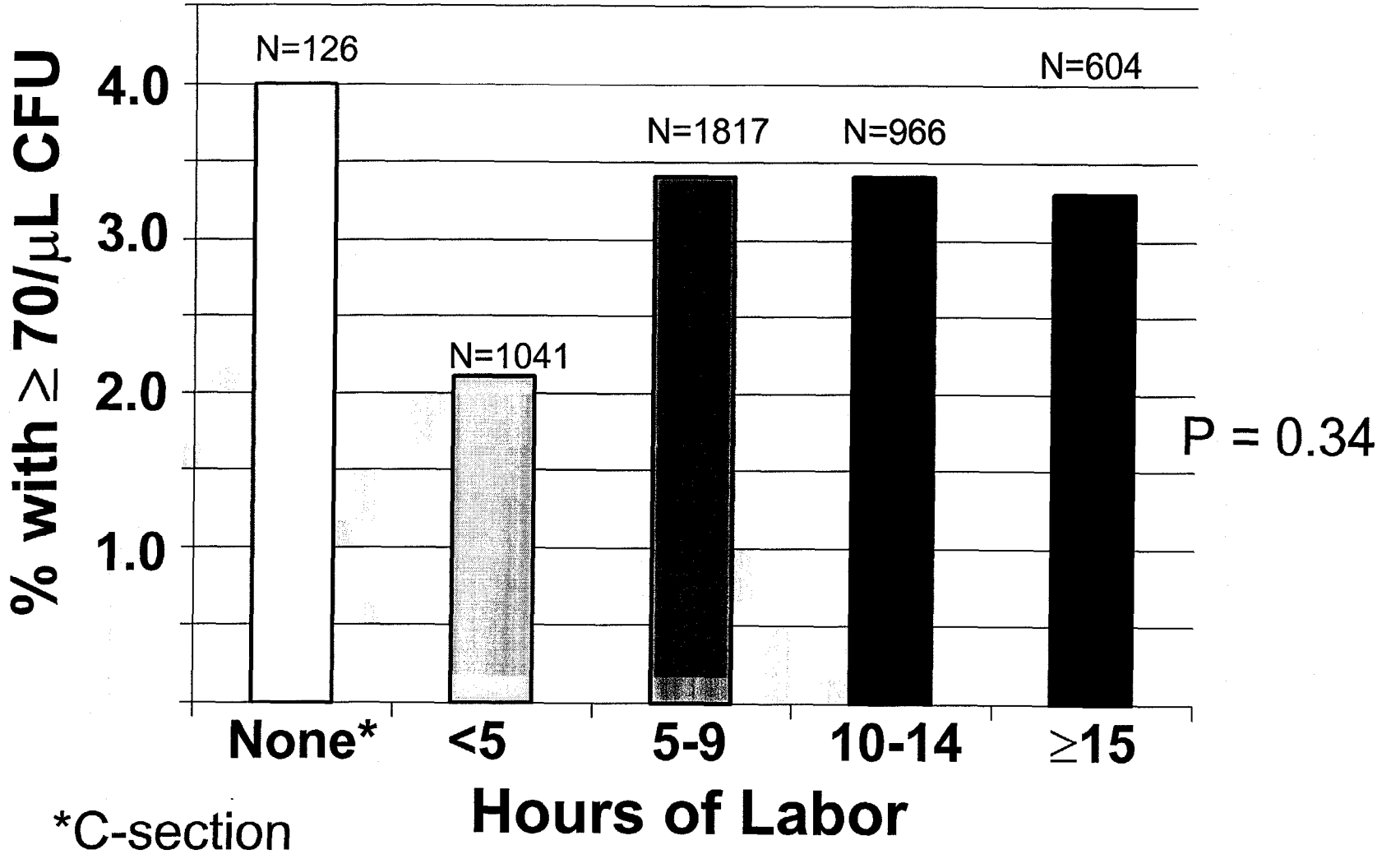


*L. Luzzatto

If NRBCs correlates with CFU, do same the same risk factors correlate with CFU?

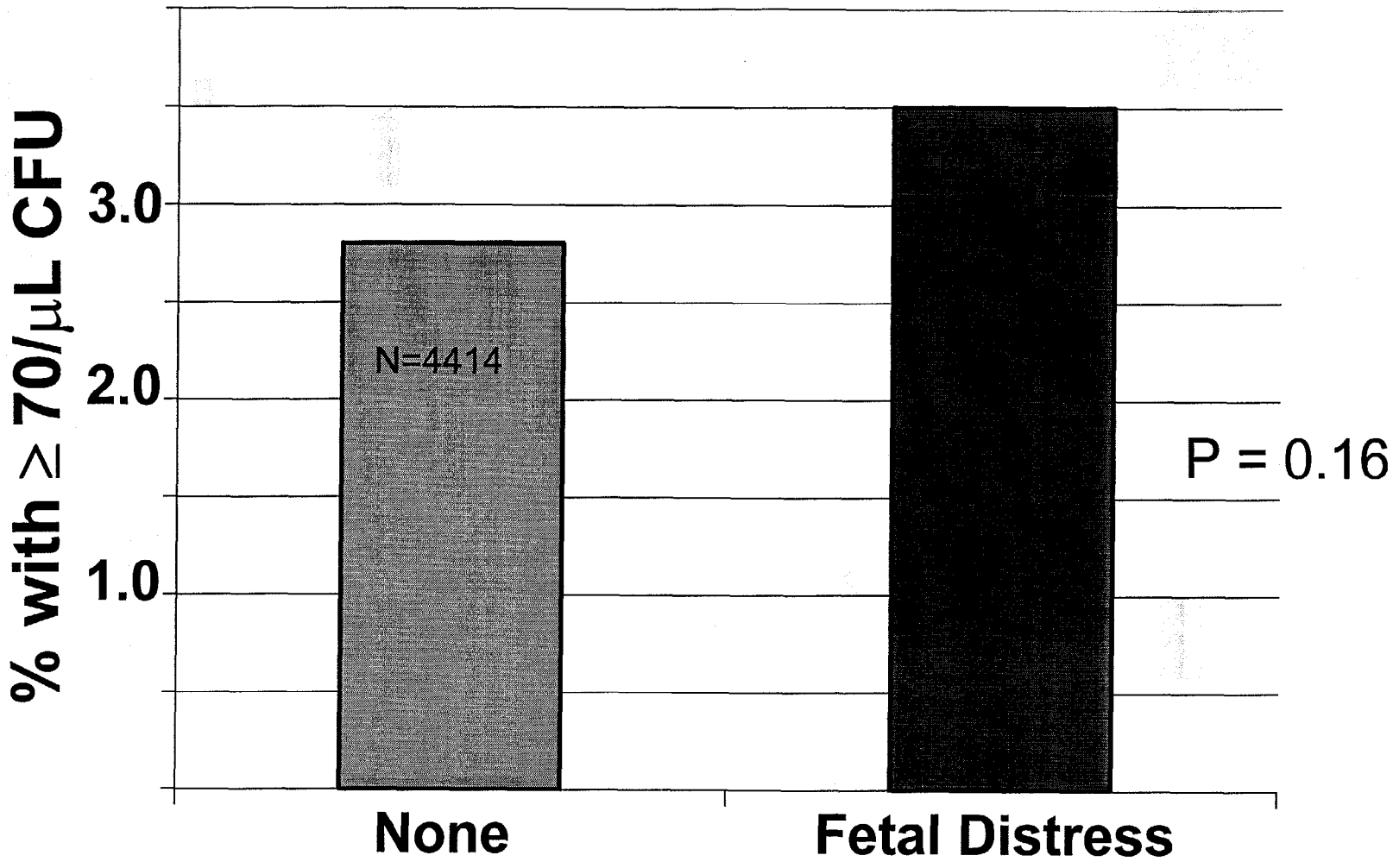

CFU ≥ 70/μL
and ABO Incompatibility
% with ≥ 70/μL CFU
5.0
4.0
3.0
2.0
1.0
N=3063
N=499
N=331
P = 0.02
None
Anti-A
Anti-B
Maternal/Infant ABO Incompatibility

CFU ≥ 70/μL and Length of Labor

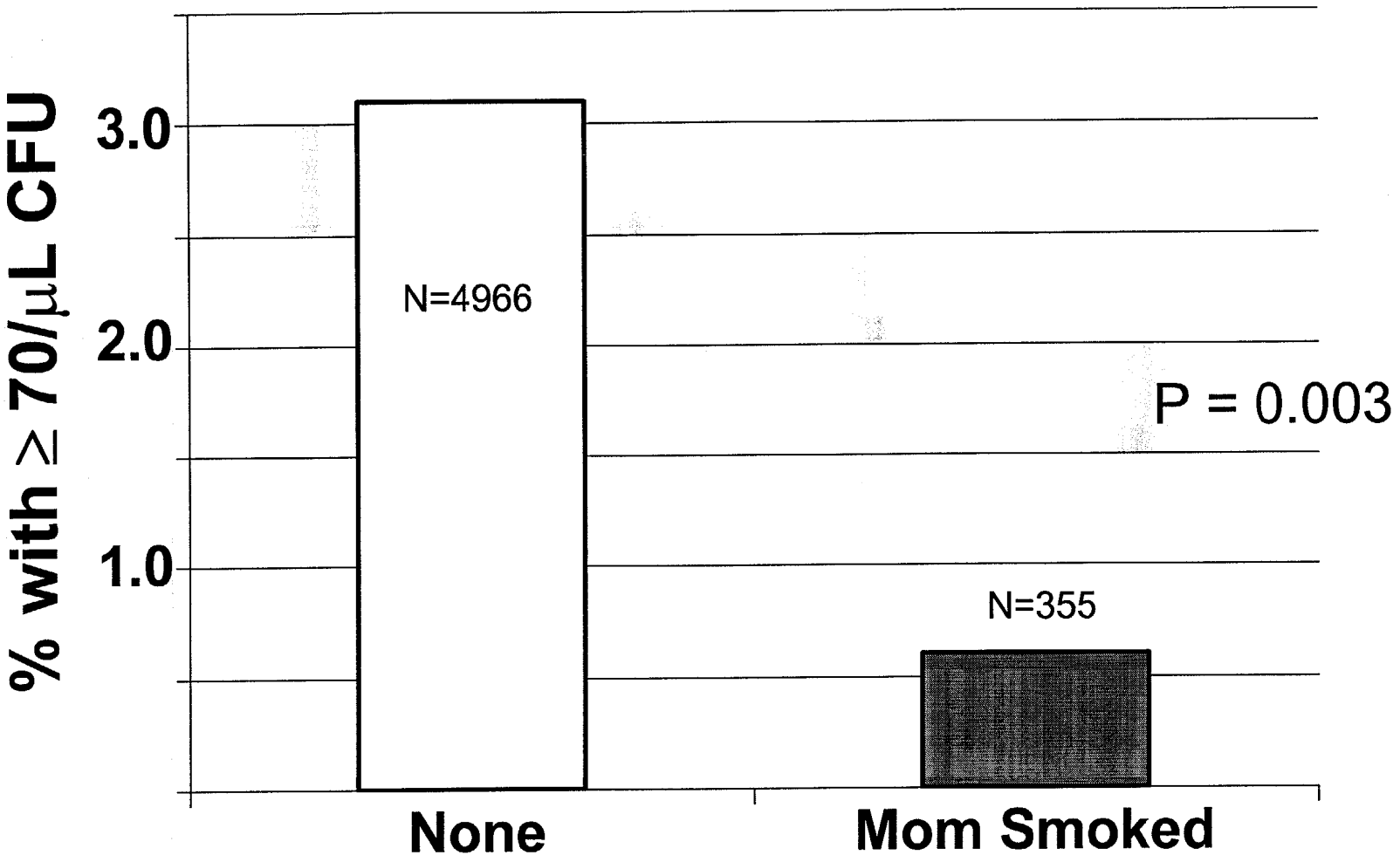


*C-section

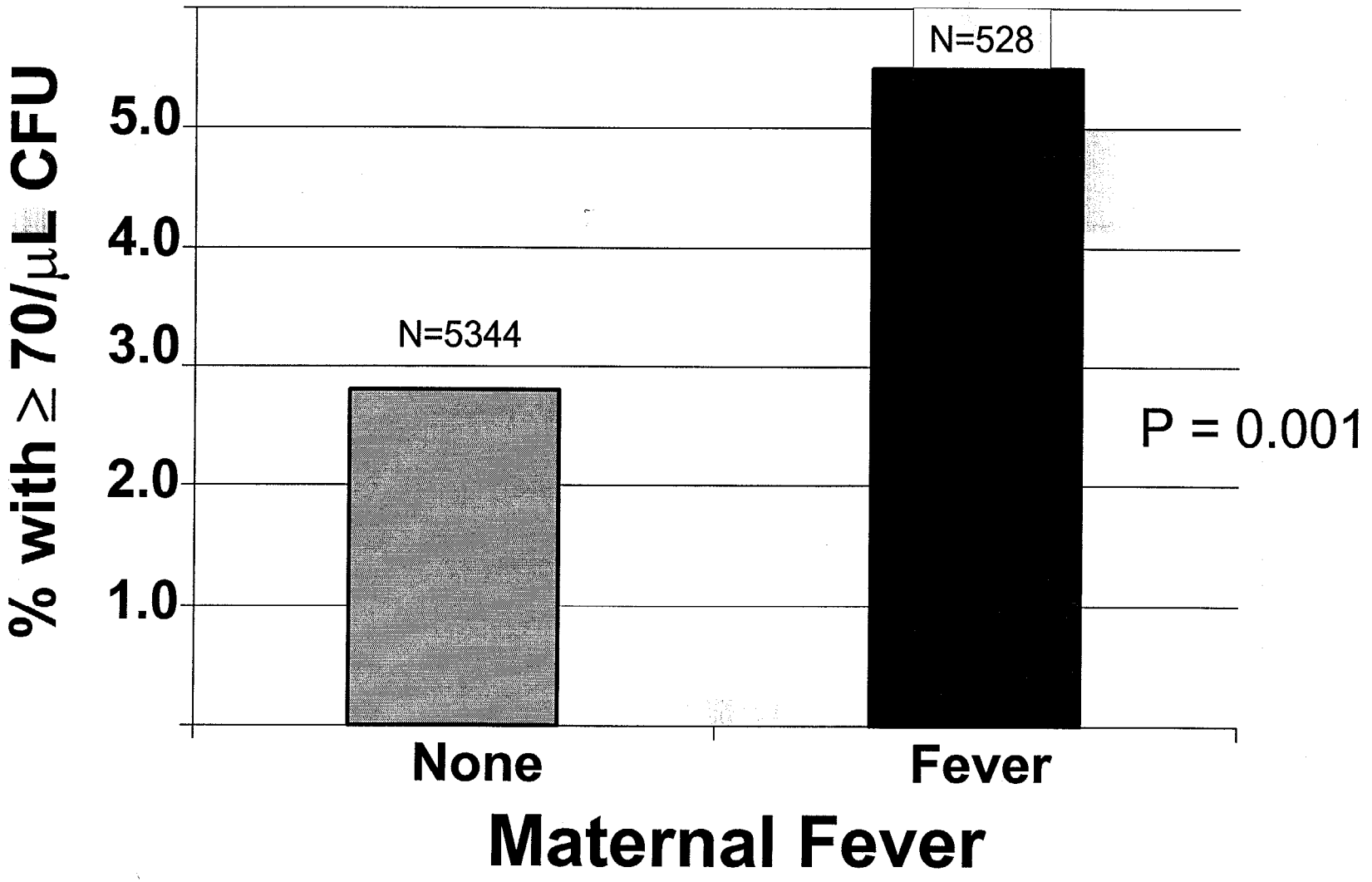
CFU ≥ 70/μL and Fetal Distress

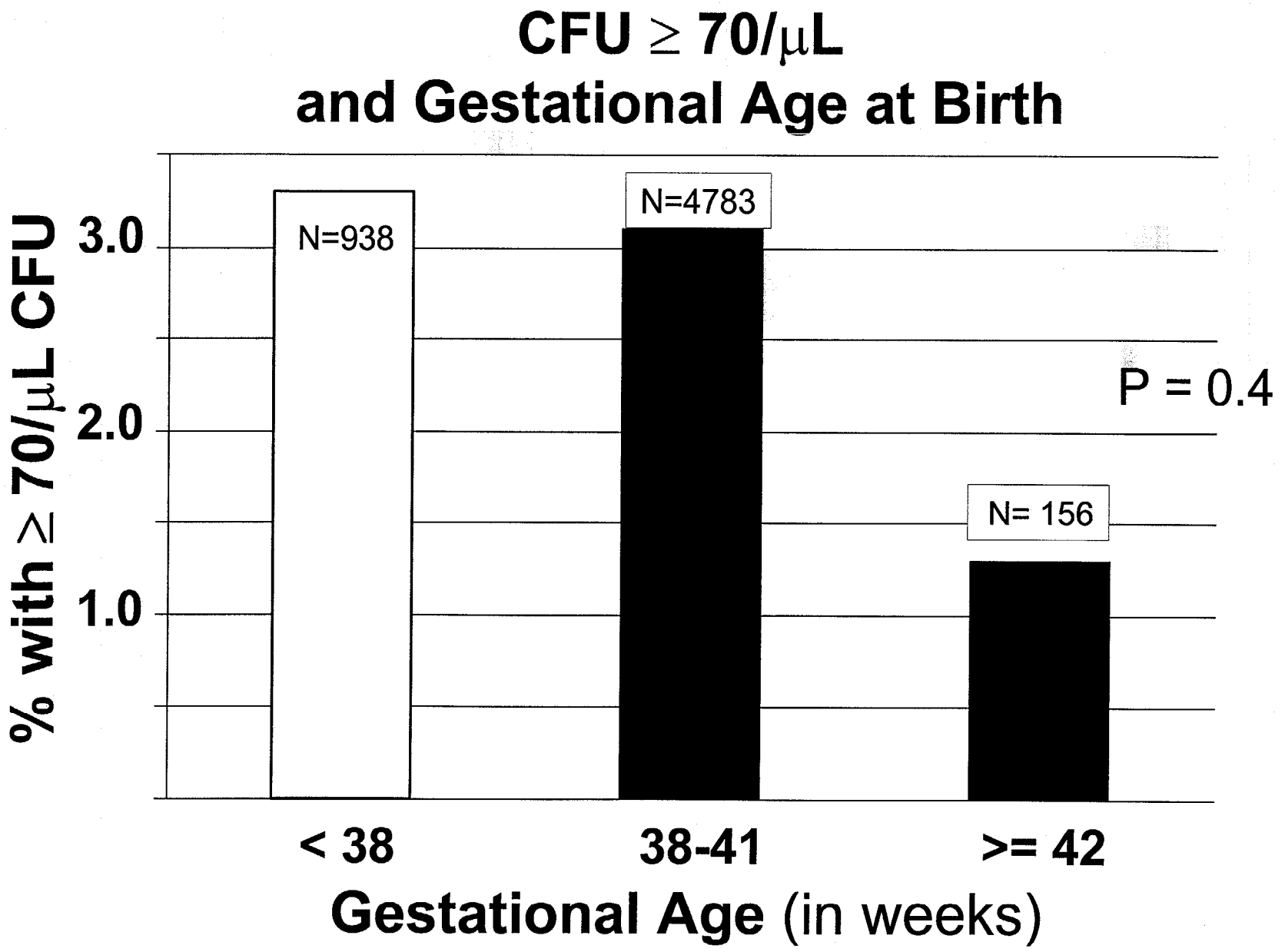


CFU ≥ 70/μL and Maternal Smoking During Pregnancy

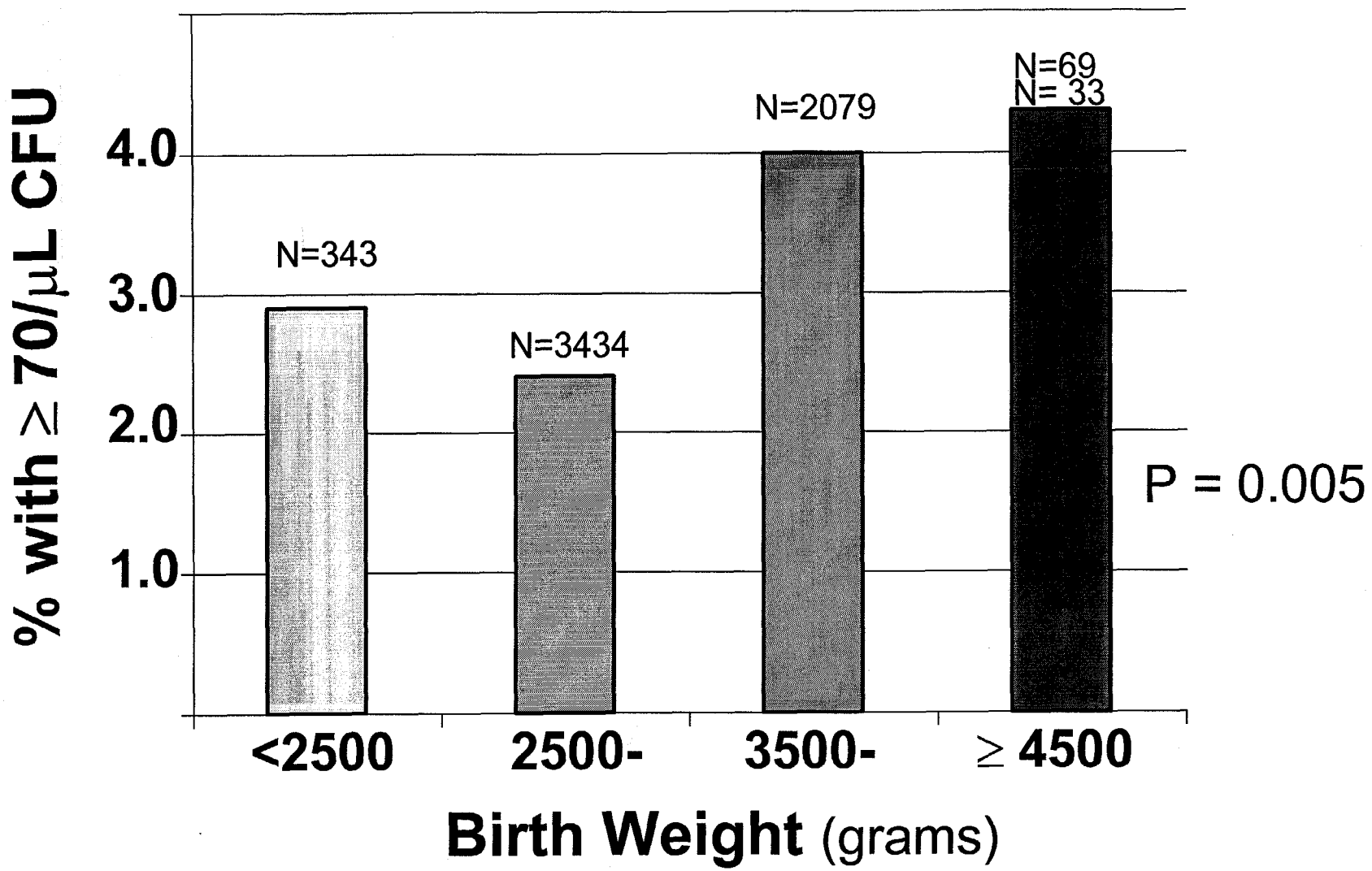


CFU ≥ 70/μL and Fever during Labor and Delivery

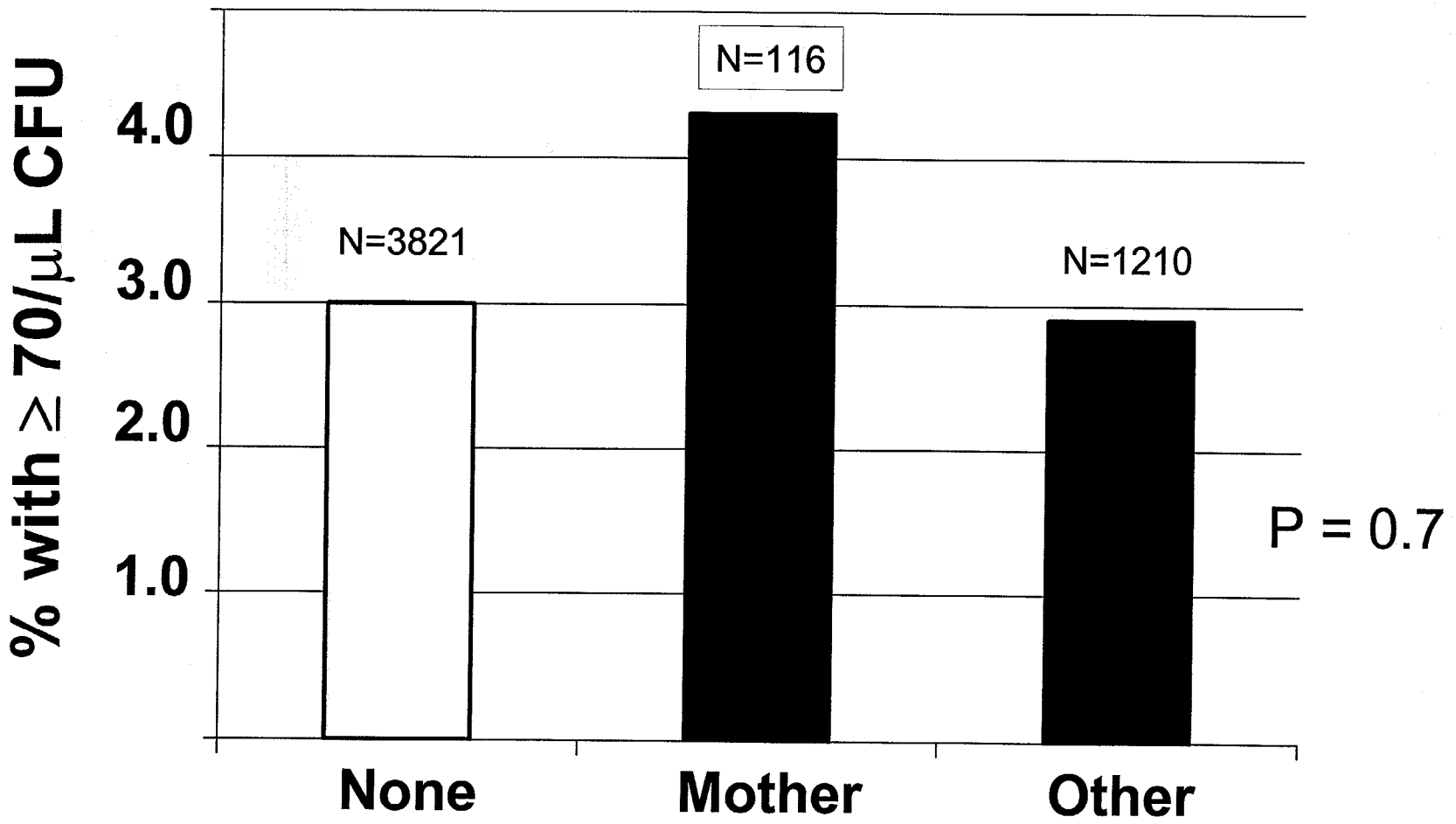



CFU ≥ 70/μL
and Gestational Age at Birth
% with ≥ 70/μL CFU
3.0
2.0
1.0
N=938
N=4783
N= 156
P = 0.4
< 38
38-41
>= 42
Gestational Age (in weeks)

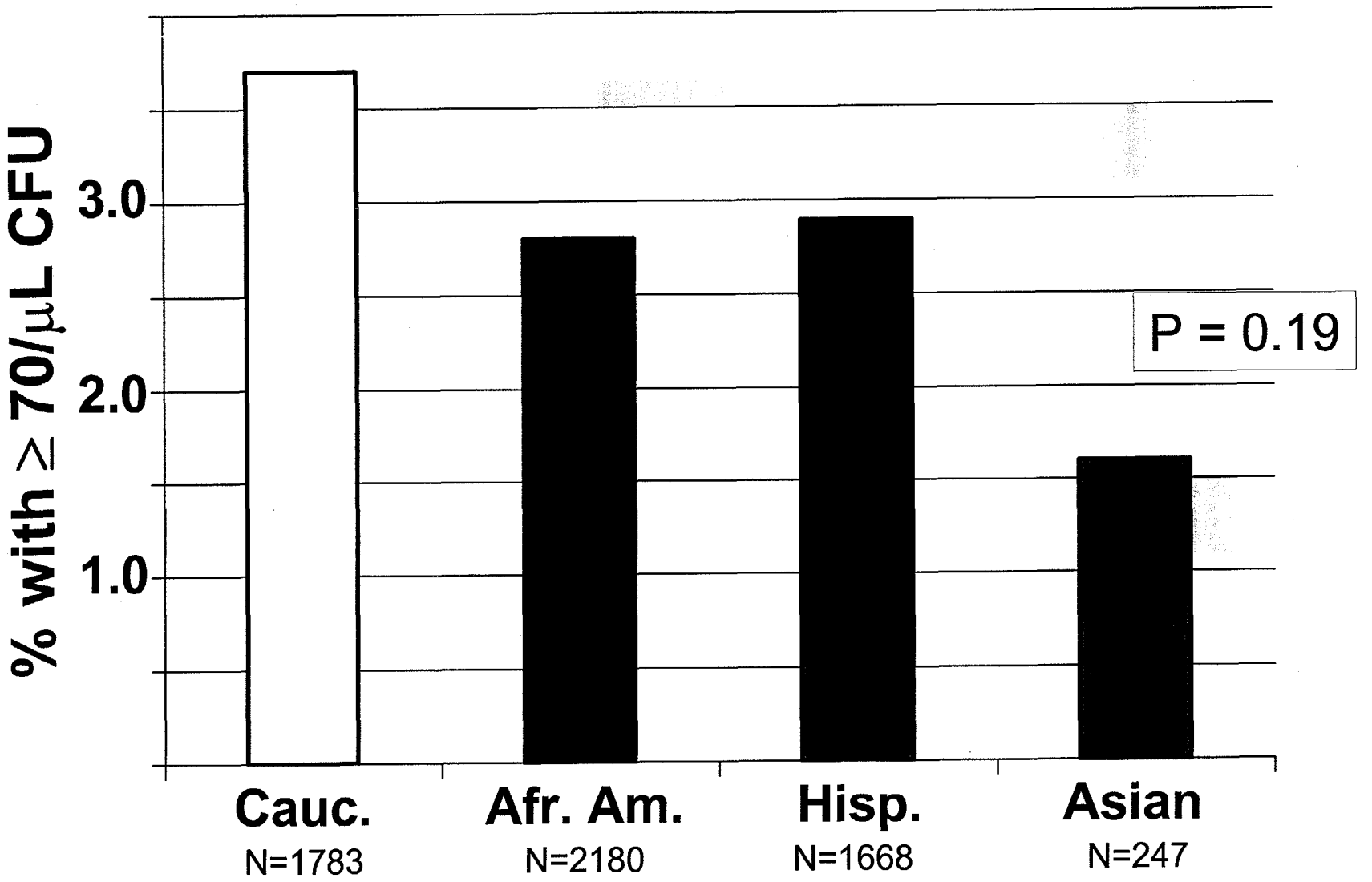
CFU ≥ 70/μL and Birth Weight



CFU ≥ 70/μL and Family History of Diabetes Mellitus



CFU ≥ 70/μL and Maternal Ethnicity

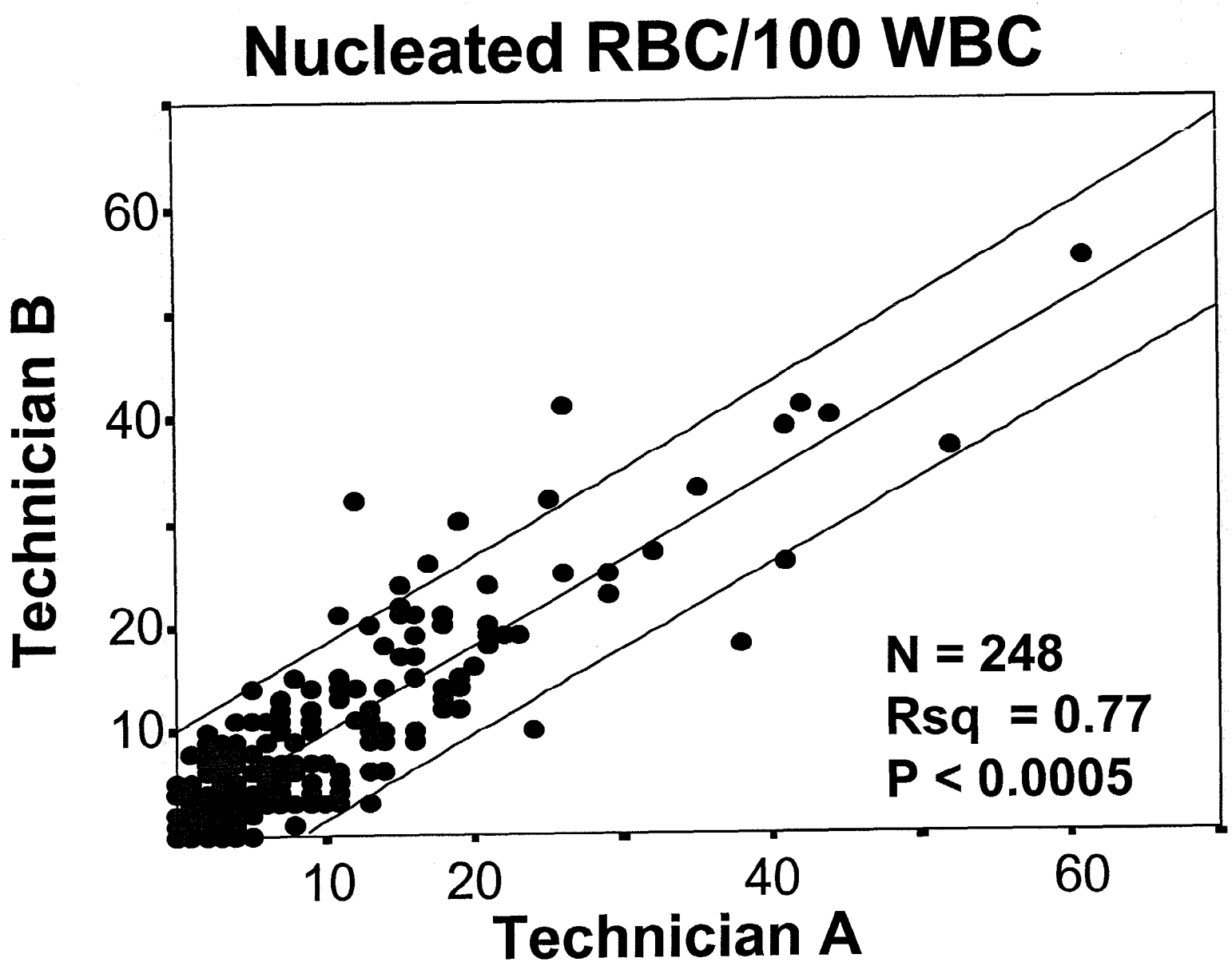


Multivariate Analysis of Factors Associated with CFU Count

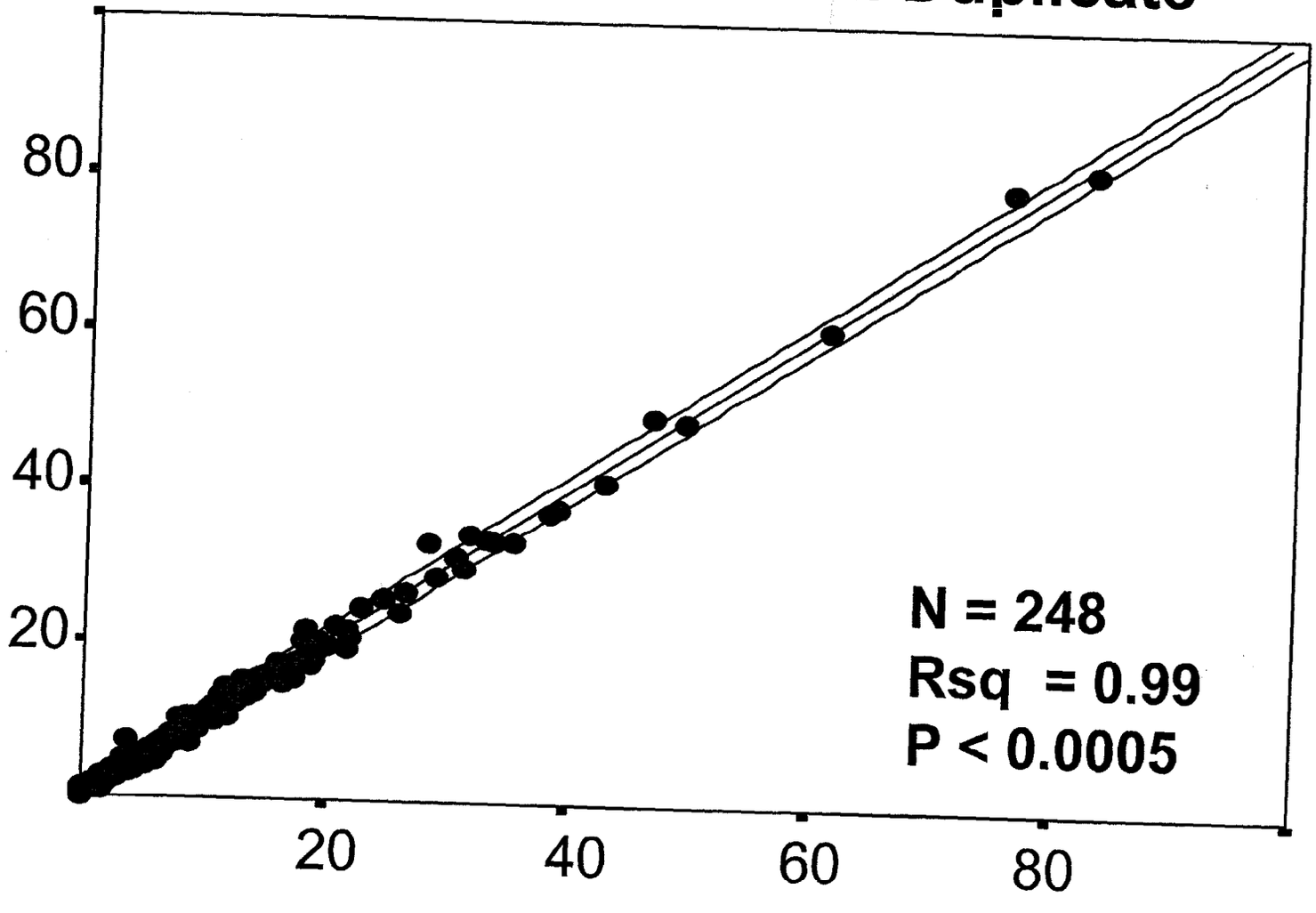
Variable	Odds Ratio*	P value
Ethnicity: Asian	-0.6 (-4 – 3)	0.7
African-Amer.	**-4.4 (-6 - -3)**	**<0.0005**
Hispanic	-2.2 (-4 – -0.6)	0.006
ABO Incomp.: A	1.9 (.2 – 4)	0.029
B	3.4 (1 - 5)	0.001
Fetal Distress	**0.7 (-.6 - 2)**	**0.3**
Maternal Fever	3.8 (2 - 6)	<0.0005
Birth Weight (in grams)	0.003 (.002 -.004)	<0.0005

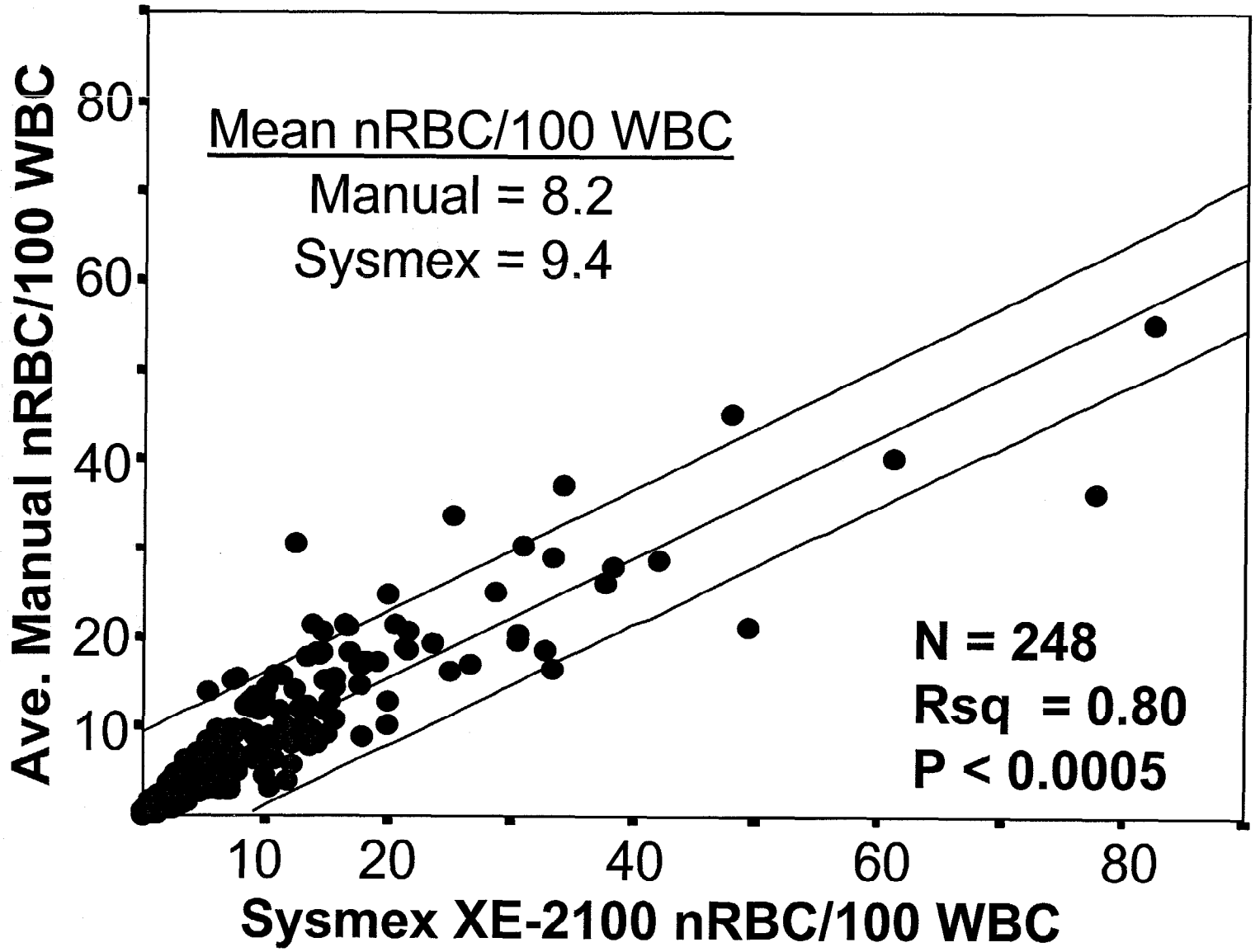
Diabetes, Apgar, Length of Labor not independently predictive of CFU count.

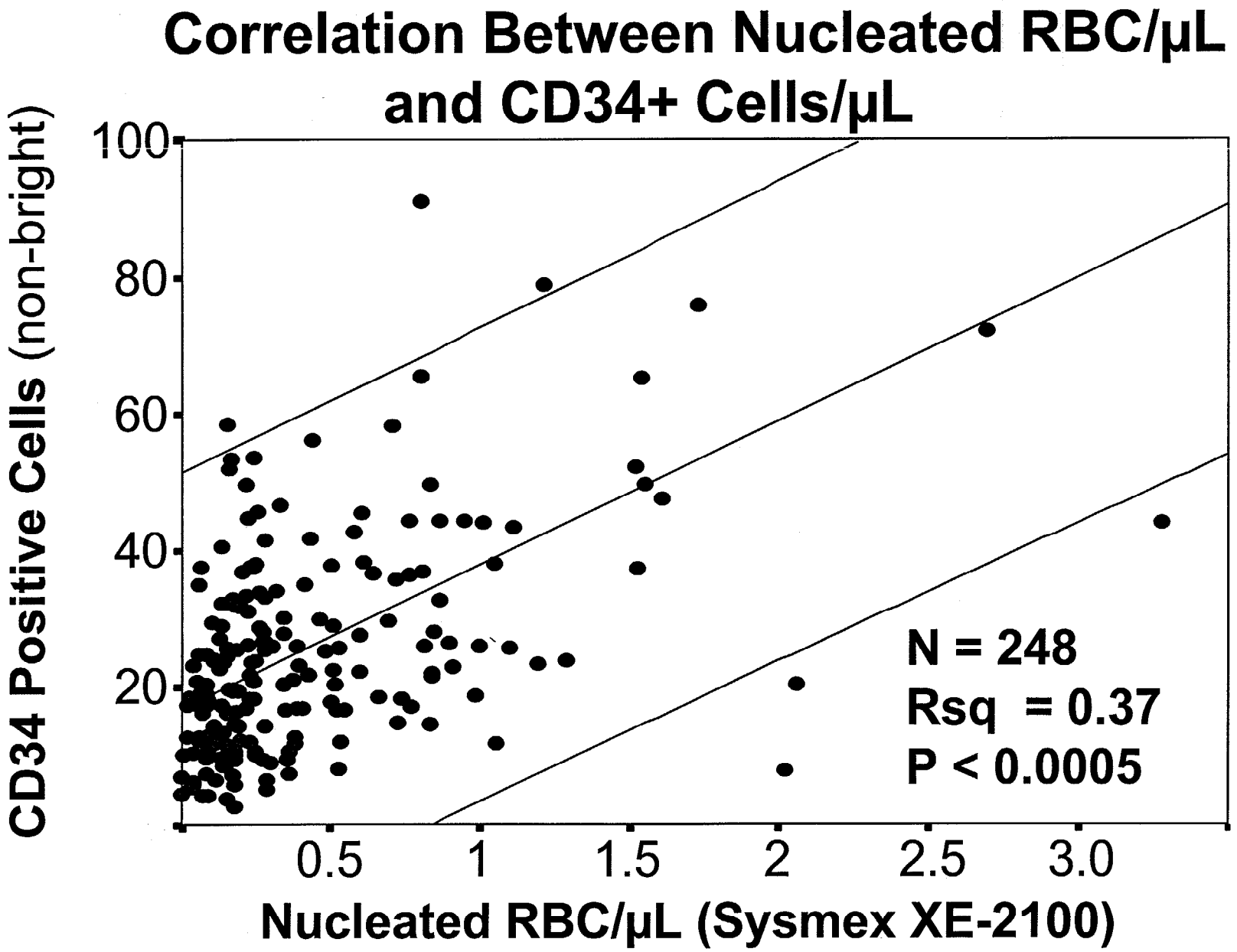
* By Linear Regression, ( ) = 95%ile.
Reference = Others in the Group


Nucleated RBC/100 WBC
Technician B
60
40
20
10
10
20
40
60
Technician A
N = 248
Rsq = 0.77
P < 0.0005

Nucleated RBC/100 WBC
By Sysmex XE-2100 in Duplicate




Mean nRBC/100 WBC
Manual = 8.2
Sysmex = 9.4
N = 248
Rsq = 0.80
P < 0.0005
Ave. Manual nRBC/100 WBC
Sysmex XE-2100 nRBC/100 WBC
10
20
40
60
80


Correlation Between Nucleated RBC/µL and CD34+ Cells/µL
CD34 Positive Cells (non-bright)
100
80
60
40
20
0.5
1
1.5
2
2.5
3.0
Nucleated RBC/µL (Sysmex XE-2100)
N = 248
Rsq = 0.37
P < 0.0005