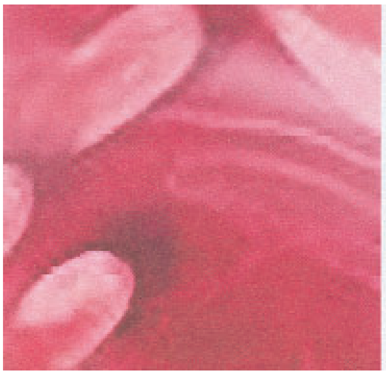


XVI Reunião da Sociedade Portuguesa de Hemorreologia e Microcirculação

"HEMORREOLOGIA, MICROCIRCULAÇÃO E *STRESS* OXIDATIVO"

26 de Novembro de 2005
Faculdade de Medicina da Universidade de Coimbra

Luís Belo

XVI Reunião da SPHM
"Hemorreologia, microcirculação e stress oxidativo"

Faculdade de Medicina Universidade de Coimbra

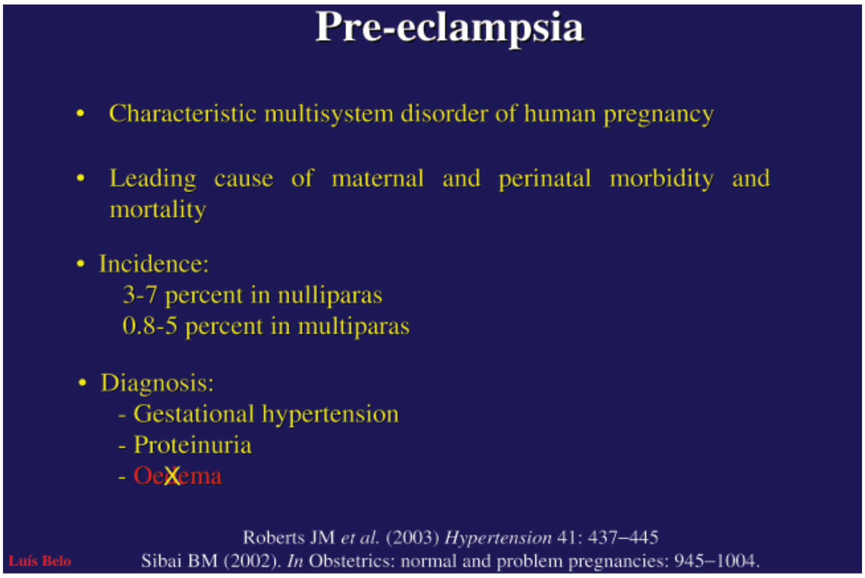
26 de Novembro de 2005

Luís Belo

IBMC

U.PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

***Stress* oxidativo na pré-eclampsia**

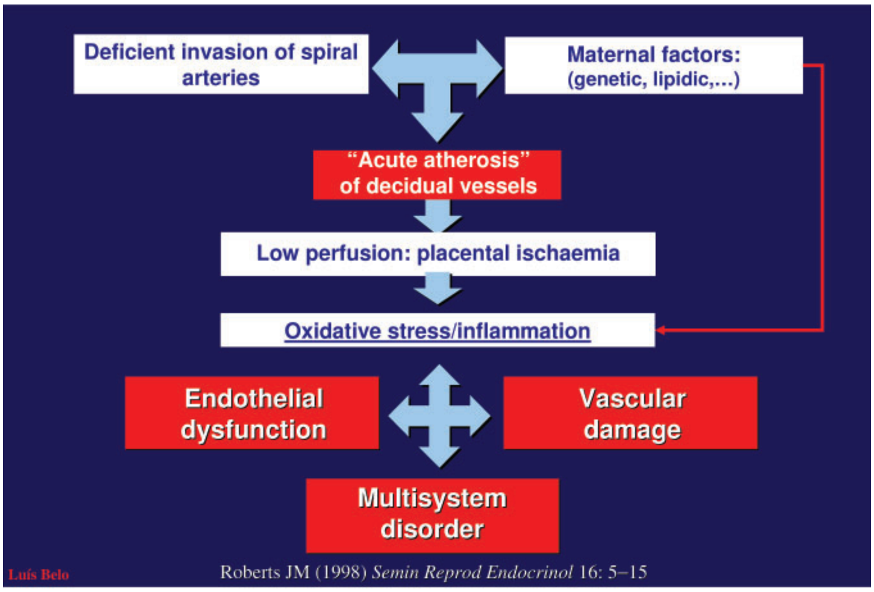


Pre-eclampsia

- Characteristic multisystem disorder of human pregnancy
- Leading cause of maternal and perinatal morbidity and mortality
- Incidence:
  3-7 percent in nulliparas
  0.8-5 percent in multiparas
- Diagnosis:
  - Gestational hypertension
  - Proteinuria
  - OeXema

Roberts JM *et al.* (2003) *Hypertension* 41: 437–445
Sibai BM (2002). *In* Obstetrics: normal and problem pregnancies: 945–1004.

Luís Belo



Deficient invasion of spiral arteries

Maternal factors: (genetic, lipidic,...)

"Acute atherosis" of decidual vessels

Low perfusion: placental ischaemia

Oxidative stress/inflammation

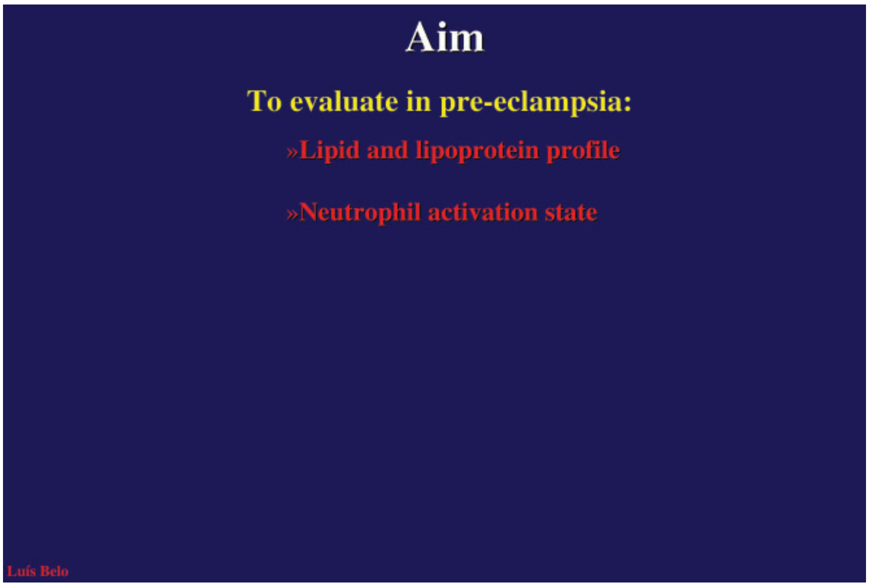
Endothelial dysfunction

Vascular damage

Multisystem disorder

Luís Belo

Roberts JM (1998) *Semin Reprod Endocrinol* 16: 5–15



Aim

To evaluate in pre-eclampsia:

»Lipid and lipoprotein profile

»Neutrophil activation state

Luís Belo

Subjects

Cross-sectional study: 3 years

(Third trimester of gestation)

»Normal pregnant women (*n* = 67)

»Pre-eclamptic women (*n* = 51)

[American College of Obstetricians and Gynecologists (ACOG) criteria]

Luís Belo

Methods

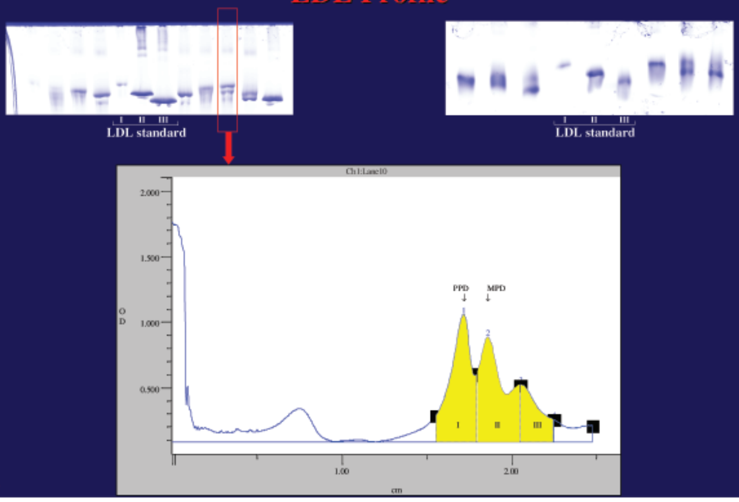
Lipids and Lipoproteins

TC	Enzymatic colorimetric tests	CHOD-PAP method, Cholesterol, ABX Diagnostics
TG		GPO-PAP method, Triglycerides, ABX Diagnostics
HDLc		HDL Cholesterol Direct, ABX Diagnostics
LDLc		LDL Cholesterol Direct, ABX Diagnostics
Apo A-I	Immunoturbidimetric assays	Apolipoprotein A1, ABX Diagnostics
Apo B		Apolipoprotein B, ABX Diagnostics
LDL-MPD	Gradient gel electrophoresis	- LDL (d 1.019-1.063 g/ml): ultracentrifugation -Havel et al. (1955)
LDL-PPD		- 2-16% polyacrylamide gels (Alamo Gels) -Nichols et al. (1986)
LDL subs (%)		- Densitometry and software analysis
Ox. LDL	ELISA	Oxidised LDL ELISA, Mercodia

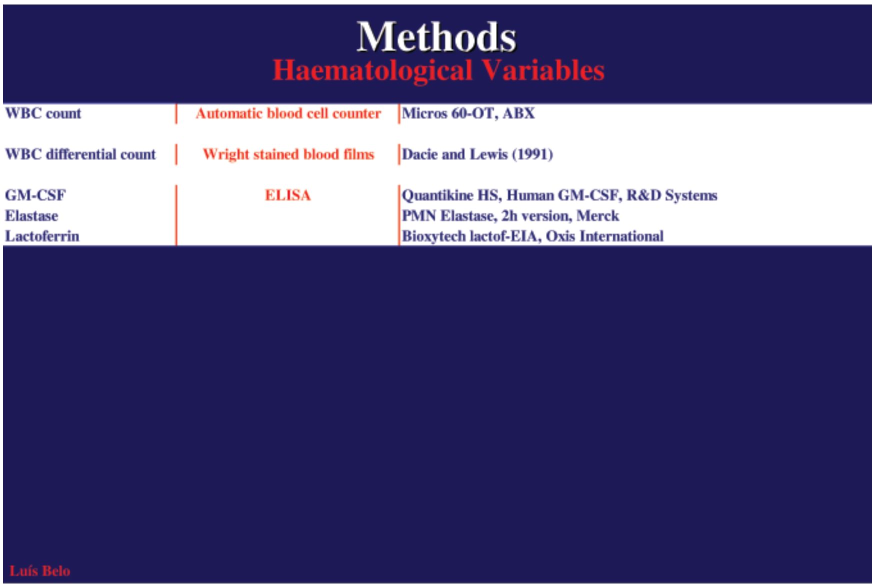
Luís Belo

Methods

LDL Profile



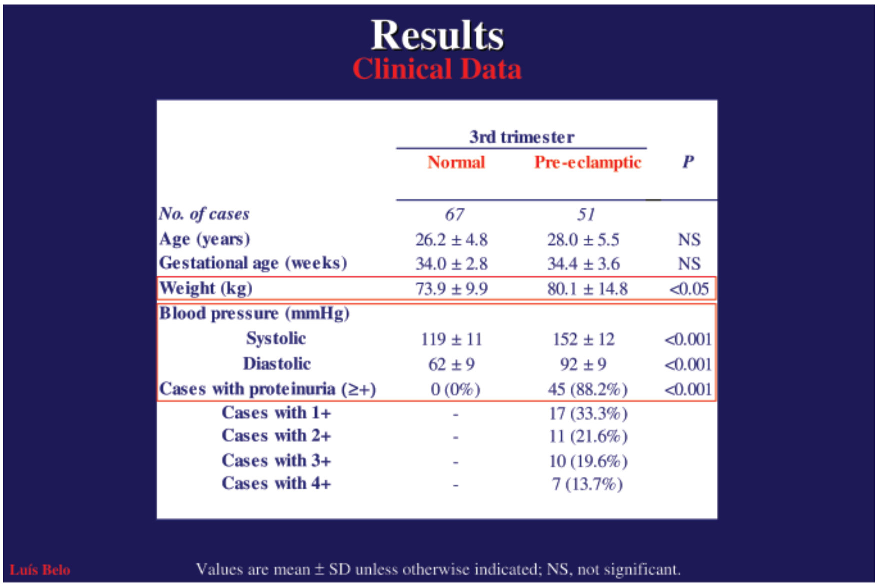
Luís Belo



Methods
Haematological Variables

WBC count	Automatic blood cell counter	Micros 60-OT, ABX
WBC differential count	Wright stained blood films	Dacie and Lewis (1991)
GM-CSF Elastase Lactoferrin	ELISA	Quantikine HS, Human GM-CSF, R&D Systems PMN Elastase, 2h version, Merck Bioxytech lactof-EIA, Oxis International

Luís Belo

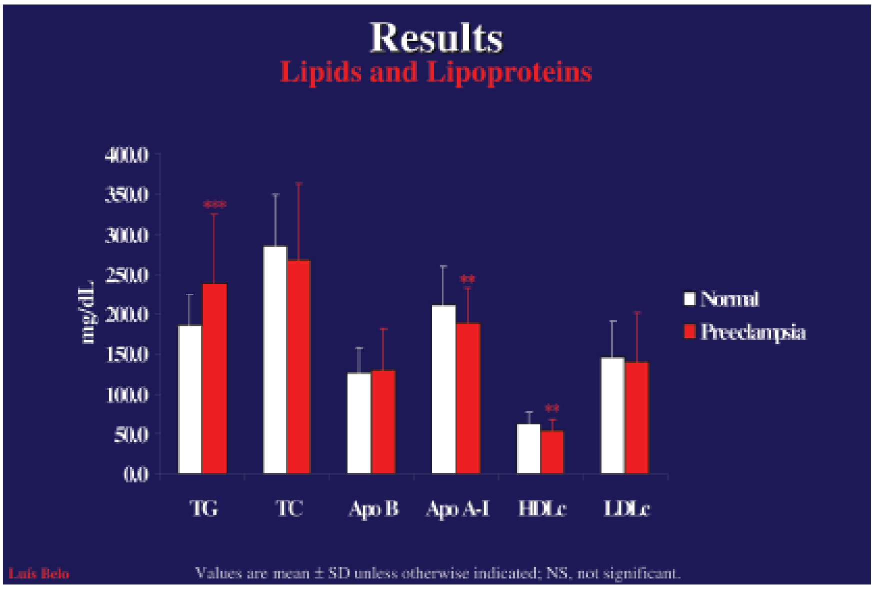


Results
Clinical Data

	3rd trimester		
	Normal	Pre-eclamptic	*P*
No. of cases	*67*	*51*	
Age (years)	26.2 ± 4.8	28.0 ± 5.5	NS
Gestational age (weeks)	34.0 ± 2.8	34.4 ± 3.6	NS
Weight (kg)	73.9 ± 9.9	80.1 ± 14.8	<0.05
Blood pressure (mmHg)			
Systolic	119 ± 11	152 ± 12	<0.001
Diastolic	62 ± 9	92 ± 9	<0.001
Cases with proteinuria (≥+)	0 (0%)	45 (88.2%)	<0.001
Cases with 1+	-	17 (33.3%)	
Cases with 2+	-	11 (21.6%)	
Cases with 3+	-	10 (19.6%)	
Cases with 4+	-	7 (13.7%)	

Luís Belo

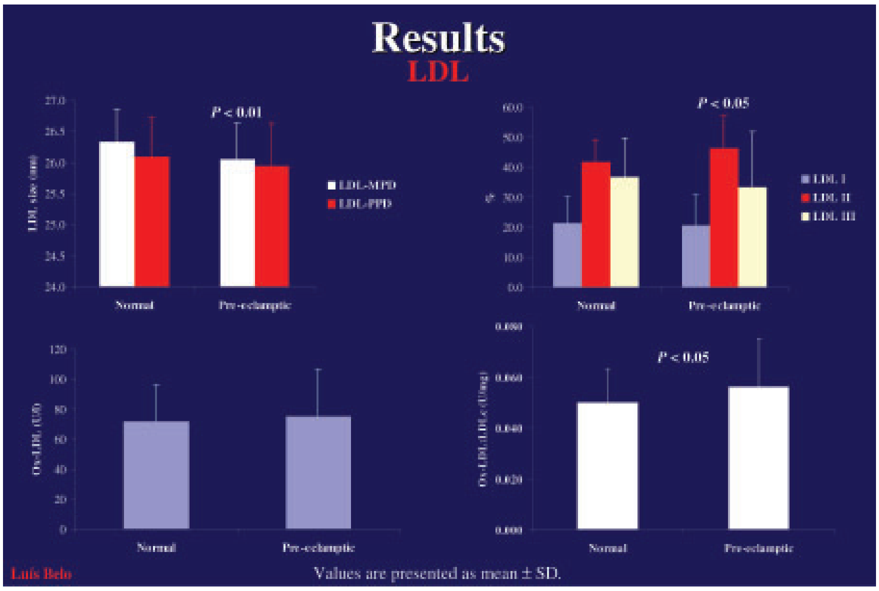
Values are mean ± SD unless otherwise indicated; NS, not significant.



Results
Lipids and Lipoproteins

Luís Belo

Values are mean ± SD unless otherwise indicated; NS, not significant.

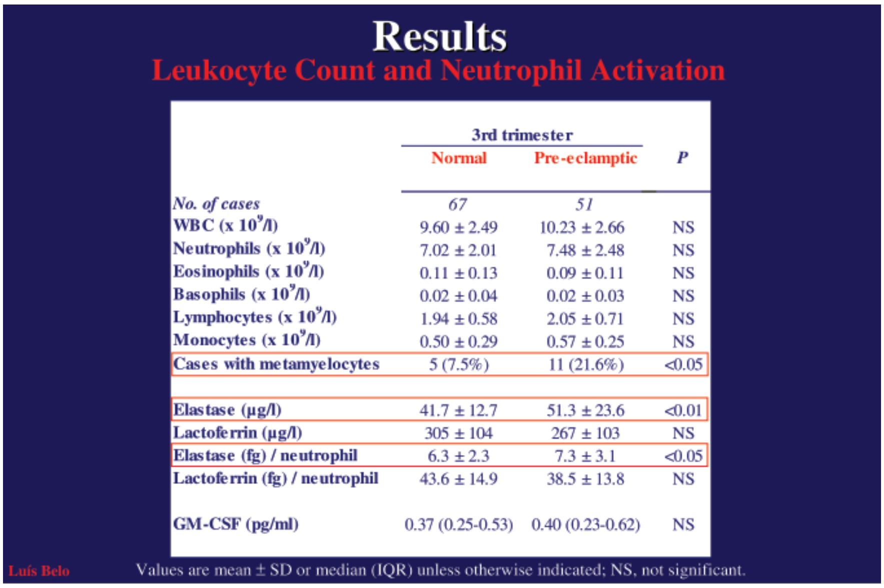


Results

LDL

Luís Belo

Values are presented as mean ± SD.



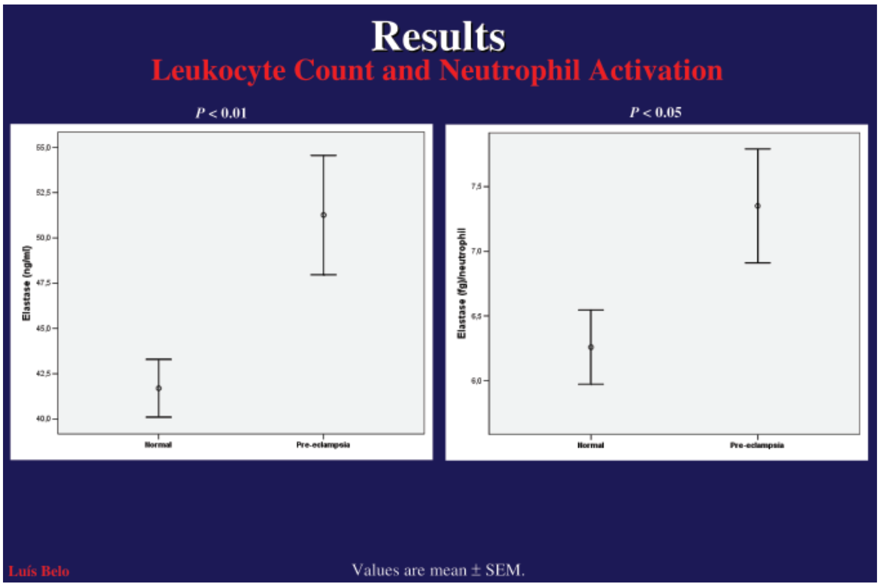
Results

Leukocyte Count and Neutrophil Activation

	3rd trimester		
	Normal	Pre-eclamptic	*P*
No. of cases	*67*	*51*	
WBC (x 10^9/l)	9.60 ± 2.49	10.23 ± 2.66	NS
Neutrophils (x 10^9/l)	7.02 ± 2.01	7.48 ± 2.48	NS
Eosinophils (x 10^9/l)	0.11 ± 0.13	0.09 ± 0.11	NS
Basophils (x 10^9/l)	0.02 ± 0.04	0.02 ± 0.03	NS
Lymphocytes (x 10^9/l)	1.94 ± 0.58	2.05 ± 0.71	NS
Monocytes (x 10^9/l)	0.50 ± 0.29	0.57 ± 0.25	NS
Cases with metamyelocytes	5 (7.5%)	11 (21.6%)	<0.05
Elastase (μg/l)	41.7 ± 12.7	51.3 ± 23.6	<0.01
Lactoferrin (μg/l)	305 ± 104	267 ± 103	NS
Elastase (fg) / neutrophil	6.3 ± 2.3	7.3 ± 3.1	<0.05
Lactoferrin (fg) / neutrophil	43.6 ± 14.9	38.5 ± 13.8	NS
GM-CSF (pg/ml)	0.37 (0.25-0.53)	0.40 (0.23-0.62)	NS

Luís Belo

Values are mean ± SD or median (IQR) unless otherwise indicated; NS, not significant.

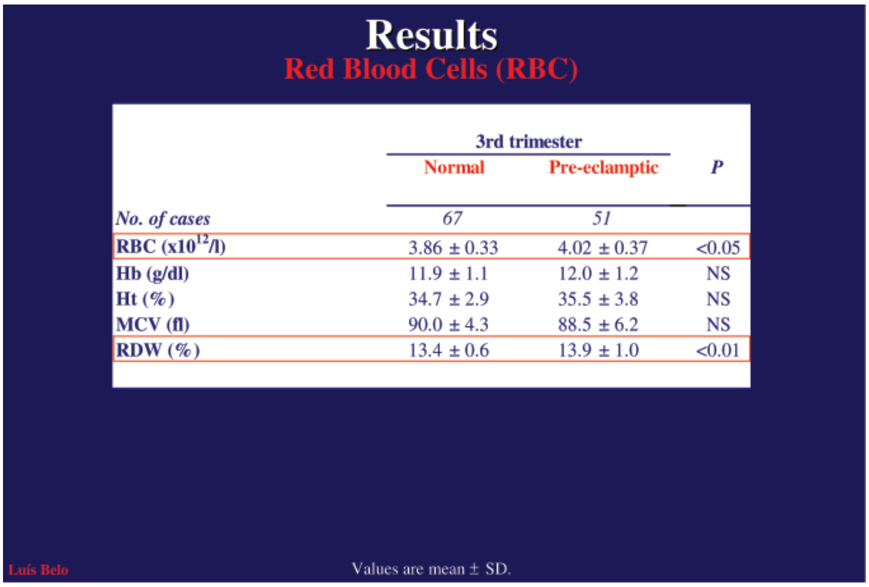


Results

Leukocyte Count and Neutrophil Activation

Luís Belo

Values are mean ± SEM.

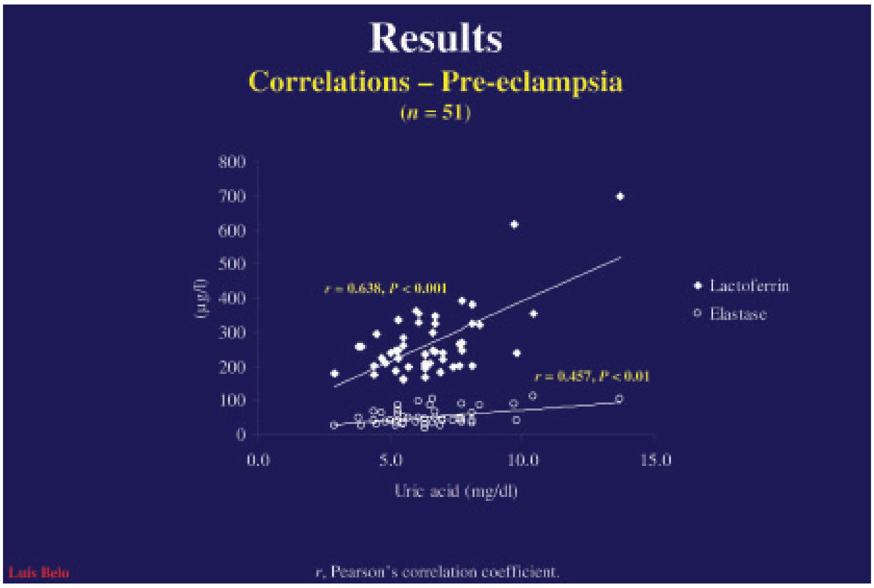


Results
Red Blood Cells (RBC)

	3rd trimester		
	Normal	Pre-eclamptic	*P*
No. of cases	*67*	*51*	
RBC (x10^{12}/l)	3.86 ± 0.33	4.02 ± 0.37	<0.05
Hb (g/dl)	11.9 ± 1.1	12.0 ± 1.2	NS
Ht (%)	34.7 ± 2.9	35.5 ± 3.8	NS
MCV (fl)	90.0 ± 4.3	88.5 ± 6.2	NS
RDW (%)	13.4 ± 0.6	13.9 ± 1.0	<0.01

Luís Belo

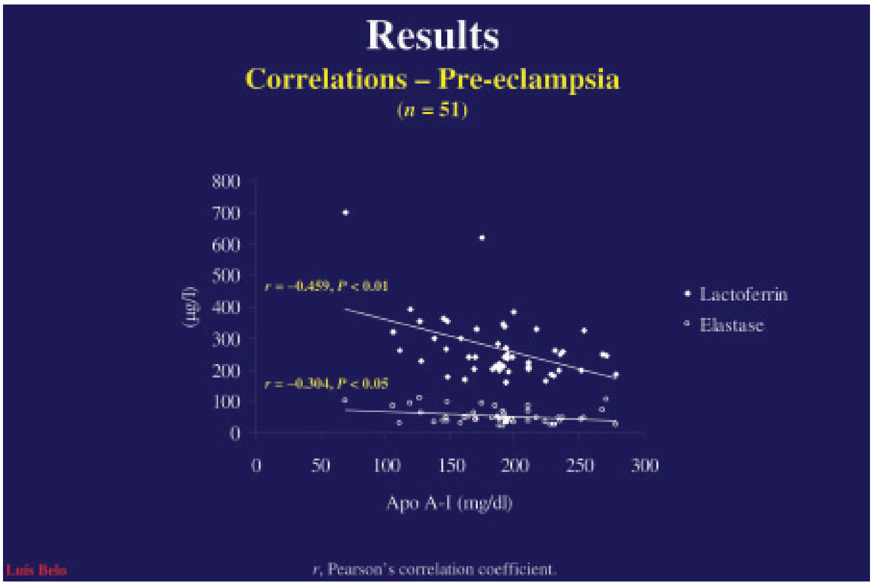
Values are mean ± SD.



Results
Correlations – Pre-eclampsia
(*n* = 51)

Luís Belo

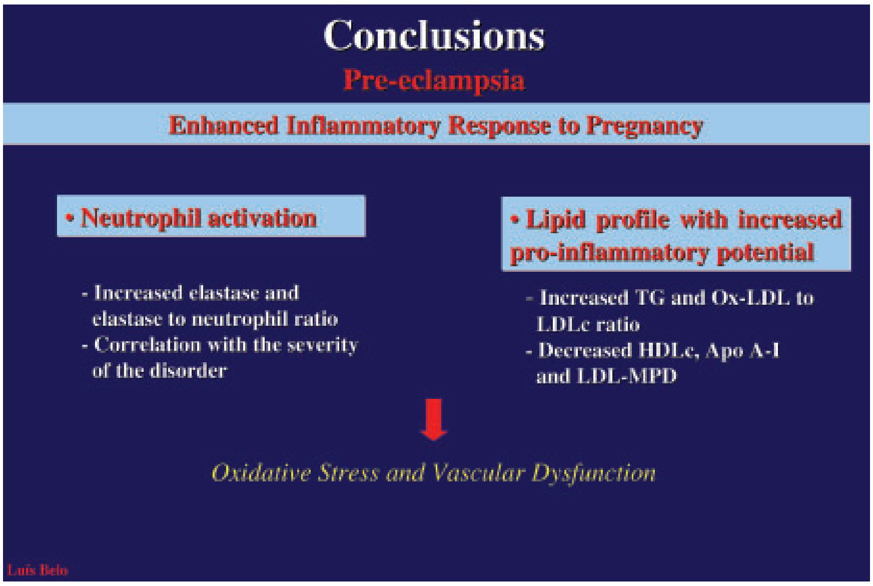
r, Pearson's correlation coefficient.



Results
Correlations – Pre-eclampsia
(*n* = 51)

Luís Belo

r, Pearson's correlation coefficient.



Conclusions

Pre-eclampsia

Enhanced Inflammatory Response to Pregnancy

• Neutrophil activation

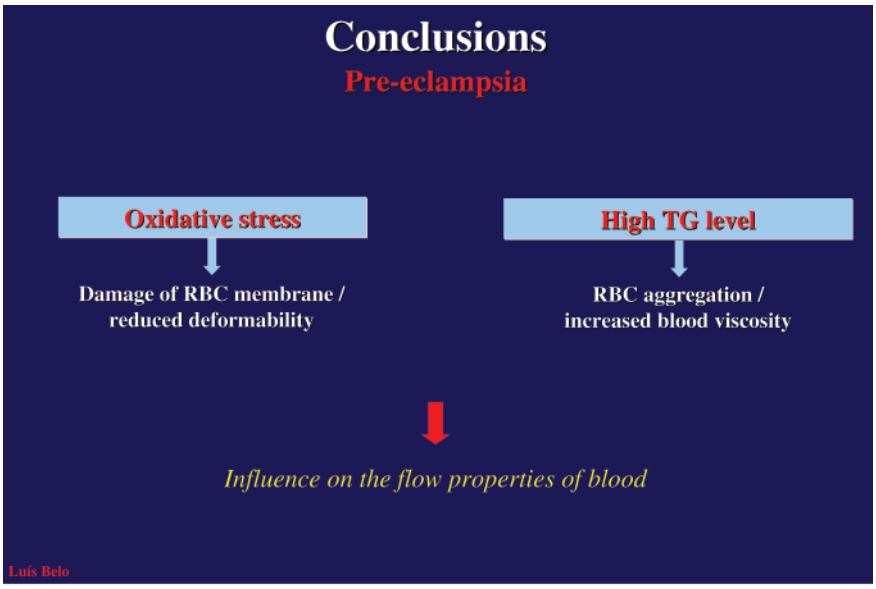
- Increased elastase and elastase to neutrophil ratio
- Correlation with the severity of the disorder

• Lipid profile with increased pro-inflammatory potential

- Increased TG and Ox-LDL to LDLc ratio
- Decreased HDLc, Apo A-I and LDL-MPD

Oxidative Stress and Vascular Dysfunction

Luís Belo



Conclusions

Pre-eclampsia

Oxidative stress

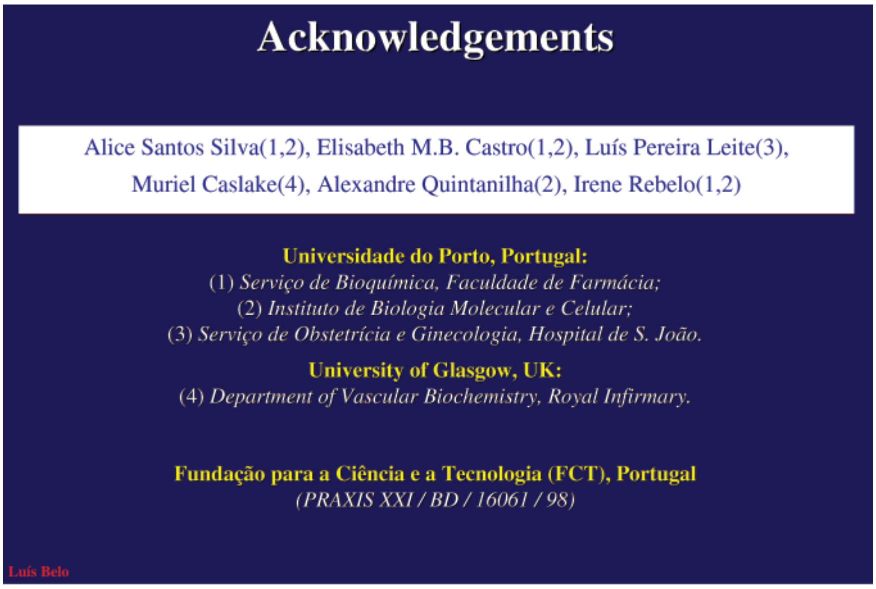
Damage of RBC membrane / reduced deformability

High TG level

RBC aggregation / increased blood viscosity

Influence on the flow properties of blood

Luís Belo



Acknowledgements

Alice Santos Silva(1,2), Elisabeth M.B. Castro(1,2), Luís Pereira Leite(3), Muriel Caslake(4), Alexandre Quintanilha(2), Irene Rebelo(1,2)

Universidade do Porto, Portugal:
(1) *Serviço de Bioquímica, Faculdade de Farmácia;*
(2) *Instituto de Biologia Molecular e Celular;*
(3) *Serviço de Obstetrícia e Ginecologia, Hospital de S. João.*

University of Glasgow, UK:
(4) *Department of Vascular Biochemistry, Royal Infirmary.*

Fundação para a Ciência e a Tecnologia (FCT), Portugal
(PRAXIS XXI / BD / 16061 / 98)

Luís Belo