CHAPTER 3

RESULTS

The primary purpose of this study was to investigate the expression of the H-subunit and L-subunit of ferritin in the bone marrow macrophage and cells of the erythron during chronic immune stimulation. In addition to this, various humoral factors were determined to assess the immune and iron status of the patients. In order to perform the H-subunit and L-subunit determinations blind, the diagnosis, immune and iron status of the patients were unknown to the investigator. The confounding factor for the inclusion of patients in this study was a bone marrow biopsy. Biopsies could, in line with the ethical clearance prescriptions for this study, only be obtained during a scheduled bone marrow biopsy for diagnostic purposes or during elective hip replacement surgery. Forty-eight patients attending the Department of Internal Medicine, Kalafong Hospital for treatment of chronic diseases, with a high prevalence of human immunodeficiency virus (HIV) infection were included in the study. Bone marrow tissue was obtained from the 48 patients during the scheduled bone marrow biopsy. All patients gave informed consent for the use of these samples including 25 ml of blood. Ethical clearance for the study was obtained from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (ethical clearance number 118/2003). Ten patients scheduled for hip replacement at the Department of Orthopaedics at the Pretoria Academic Hospital were included in the study as a group of patients with less severe immune stimulation. These patients were all diagnosed with osteoarthritis and were HIV-negative. Bone marrow tissue from the osteoarthritis patients was taken during hip replacement surgery.

All patients gave informed consent for the use of these samples including 25 ml of blood. Ethical clearance for the study was obtained from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (ethical clearance number 285/2003).

Table 1 shows the demographics of the patients from the Department of Internal Medicine and from the Department of Orthopaedics. Table 2 shows the diagnosis and HIV status of the patients from the Department of Internal Medicine. The diagnosis of the patients from the Department of Internal Medicine was diverse and included various types of infections (tuberculosis (TB), malaria, HIV), cancers (lung, breast), pancytopenias as a result of bone marrow suppression or peripheral destruction of blood cells, organ failures including renal failure, heart failure and liver failure, anaemias with different etiologies and various other pathologies that resulted in inflammatory reactions. This resulted in an extremely heterogenous group of patients. For the purpose of this study the immune status and iron status, respectively, were used to group these patients. The osteoarthritis patients were treated as a separate group.

Table 1. Demographics of the patients from the Department of Internal Medicine, Kalafong Hospital (patients 1-48) and the Department of Orthopaedics, Pretoria Academic Hospital (patients 1-10)

Patient	Age	Sex	Race
1	31	Female	Black
2	82	Female	Black
3	27	Female	Black
4	42	Female	Black
5	41	Female	Black
6	58	Female	White
7	46	Male	Black
8	72	Male	Black
9	29	Female	Black
10	22	Female	Black
11	42	Male	Black
12	54	Female	Black
13	33	Female	Black
14	44	Female	Black
15	40	Female	Black
16	25	Female	Black
17	33	Female	Black
18	36	Female	White
19	24	Female	Black
20	24	Female	Black
21	25	Female	Black
22	54	Female	White
23	31	Female	Black
24	33	Female	Black
25	47	Female	Black
26	50	Female	Black
27	18	Male	Black
28	45	Female	Black
29	62	Female	Black
30	45	Female	White
31	25	Female	Black
32	39	Female	Black
33	27	Female	Black
34	28	Female	Black
35	62	Female	Black
36	33	Female	Black
37	41	Female	Black
38	53	Female	Black
39	67	Male	Black
40	40	Male	Black
41	29	Male	Black
42	72	Male	Black
43	44	Male	Black
44	60	Female	Black
45	35	Male	Black
46	74	Female	Black
47	34	Female	Black
48	17	Female	Black

Patient	Age	Sex	Race
1	48	Female	Black
2	61	Female	White
3	63	Male	White
4	43	Female	White
5	62	Male	White
6	65	Female	White
7	77	Female	White
8	71	Male	White
9	65	Male	White
10	65	Female	White

Table 2. Diagnosis and HIV status of the patients from the Department of Internal Medicine, Kalafong Hospital

Patient	Diagnosis	HIV status
1	Pneumonia & sepsis, Escherichia coli & urinary tract infection & acute renal failure	positive
2	Heart failure & megaloblastic anaemia & pernicious	negative
3	Anaemia & idiopathic thrombocytic purpura & acute haemolytic anaemia vs disseminated intravascular coagulopathy, post mortem	positive
4	Carcinoid cancer & pneumonia & metastasis to the brain	negative
5	Idiopathic vasculitis & pancytopenia questioning antiphospholipid syndrome & uterine mass	negative
6	Malaria & idiopathic thrombocytic purpura	negative
7	Cruveilhier-Baumgarten disease & hypersplenism & TB	negative
8	Lung cancer & acute renal failure & pneumonia	negative
9	Pneumonia, Escherichia coli	positive
10	Idiopathic thrombocytic purpura & iron deficient anaemia & questioning systemic lupus erythematosus	negative
11	Retro viral disease & renal failure	positive
12	Metastatic breast cancer	negative
13	Pneumonia & confusion	positive
14	Pulmonary TB & effusion	positive
15	Megaloblastic anaemia	negative
16	Anaemia due to blood loss	negative
17	Retro viral disease & anaemia & kaposi's sarcoma & previous pulmonary TB	positive
18	Antiphospholipid syndrome & haemolytic anaemia	negative
19	Retro viral disease & bicytopenia & mycobacterium avium complex	positive
20	Retro viral disease & anaemia & dilated cardiomyopathy & antiphospholipid syndrome & TB & thrombosis	positive
21	Retro viral disease & TB & sepsis & anaemia & ascitis	positive
22	Chronic obstructive pulmonary disease & liver & kidney failure & pelagra & sepsis & ethanol abuse	negative
23	Retro viral disease & pneumonia & pancytopenia	positive
24	Idiopathic thrombocytic purpura	negative
25	Retro viral disease & diabetes mellitus & heart failure & obesity & splenomegaly & lymphadenopathy & TB, bone marrow	positive
26	Ethanol abuse & radial fracture & pulmonary TB & bradycardia & primary hypertension, increase calcium	negative
27	Massive splenomegaly & pancytopenia	negative
28	Megaloblastic anaemia & syphilis	negative
29	Delerium & TB & calcified nodes, post mortem = miliary TB	negative
30	OD & thrombocytopenia & macrocytosis & ethanol abuse	negative
31	Retro viral disease & pulmonary TB & pancytopenia	positive
32	Nephritis: hypertension, edema, proteinuria	negative
33	Pancytopenia & ascitis & TB & proteinuria & urinary tract infection & sepsis, ICU	negative
34	Retro viral disease & Hodgkin's disease & TB	positive
35	Hypertension & diabetes mellitus & leucocytosis, persistent	negative
36	Retro viral disease on HAART & pulmonary TB	positive
37	Retro viral disease & idiopathic thrombocytic purpura – immune, normal spleen	positive
38	Megaloblastic anaemia & hypothyroidism	negative
39	Anaemia	negative
40	Anaemia & pyrexia & miliary TB, bone marrow culture	positive
41	Pancytopenia & idiopathic thrombocytic purpura	positive
42	Iron deficient anaemia	negative
43	Iron deficient anaemia & peptic ulcer disease & pneumonia, Staphylococcus aureus	negative
44	Idiopathic 4-limb african gangrene	negative
45	Retro viral disease & pneumonia & pancytopenia	positive
46	Monoclonal gammopathy of undetermined significance & uterine mass	negative
47	Retro viral disease & pancytopenia & pneumonia	positive
48	Idiopathic thrombocytic purpura & bicytopenia	negative

1) Determinations of the study

1.1) The expression of the H-subunit and L-subunit in the macrophage of the bone marrow and cells of the erythron (Table 3). The electron micrographs and raw data of these determinations are contained in volume 2, chapter 6.

1.2) Serum iron markers including serum iron, transferrin, transferrin saturation, ferritin and soluble transferrin receptor (Table 4).

1.3) Red blood cell characteristics including red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width and reticulocyte production index (Table 4).

1.4) Prussian blue iron stains of bone marrow aspirates and bone marrow core biopsies. Reports by Haematologists from the National Health and Laboratory Services and photographs are contained in volume 2, chapter 6.

1.5) Evaluations of the presence or absence of an iron transfer block are contained in volume 2, chapter 6.

1.6) Cytokines including Il-1, Il-2, Il-4, Il-5, Il-6, Il-8, Il-10, Il-12, TNF-α, TGF-β, INF-γ and GM-CSF (Table 5).

1.7) Neopterin (Table 5).

1.8) C-reactive protein (Table 5).

1.9) Pro-hepcidin and caeruloplasmin (Table 5).

2) Results of the study

2.1) Expression of the H-subunit and L-subunit of ferritin in bone marrow macrophages and cells of the erythron

The expression of the H-subunit and L-subunit of ferritin in the macrophage and cells of the erythron in the bone marrow were determined for both the patients from the Department of Internal Medicine, Kalafong Hospital (Kalafong patient group) and the Department of Orthopaedics, Pretoria Academic Hospital (osteoarthritis group). The results are expressed as count/μm^2. Table 3 contains the mean gold particle count/μm^2 for the macrophage, the cells of the erythron and the H-subunit/L-subunit ratios for the macrophages and cells of the erythron.

Table 3. Expression of the H-subunit and L-subunit of ferritin in the bone marrow macrophage and cells of the erythron for the Kalafong patient group (patients 1-48) and the osteoarthritis patient group (patients 1-10)

Patient	H-subunit macrophages count/µm²	L-subunit macrophages count/µm²	H-subunit/L-subunit macrophages	H-subunit erythron count/µm²	L-subunit erythron count/µm²	H-subunit/L-subunit erythron
1	127.8	68.3	1.9	244.8	109.8	2.2
2	107.7	68	1.6	198.2	132.2	1.5
3	82.3	62.7	1.3	104.7	119.2	0.88
4	119			174		
5	128	52.3	0.4	179.3	207.4	0.86
6	104.3	66	1.6	241.2	194.3	1.2
7	146.3	136.3	1.1	257.3	176.4	1.5
8	66.3	69.8	0.95	107.7	251.2	0.43
9	160.6	129.7	1.2	263.9	261.3	1
10	95.3	150.3	0.63	232.1	227.7	1
11						
12	29	75.3	0.38	70.3	145.7	0.48
13	61.5	148.7	0.41	142.8	190.6	0.75
14	61	118.3	0.52	182.3	135.1	1.3
15	41.8	126.5	0.33	86	190.1	0.45
16	65.7	124.3	0.53	112.6	199.8	0.56
17	126	119.3	1.1	164	181.2	0.9
18	64.3	103	0.62	90.3	190.2	0.47
19	140.8	148.7	0.95	119.5	273.7	0.44
20	37.5	107.7	0.35	68.3	126.9	0.54
21	96.7	85.5	1.1	94.5	144.6	0.65
22	110	94.5	1.2	117	160.5	0.73
23						
24	43.5	57.7	0.75	87.3	134.3	0.65
25	60.3	123.8	0.49	76.5	155.8	0.49
26	64.3	190	0.34	100.1	339.9	0.29
27	76.3	118	0.65	108.2	197.4	0.55
28	58.7	230.3	0.25	127	398.8	0.32
29	64.5	190	0.34	138.1	277.3	0.5
30	54.5	166.3	0.33	104.2	355.7	0.29
31						
32	61.3	100.7	0.61	107.5	261.4	0.41
33	91.9	152	0.6	101.5	269.7	0.38
34	45	163.7	0.27	64.5	223.2	0.29
35	54.3	137.3	0.4	133.3	335.7	0.4
36	58	59.3	0.98	117.7	259.4	0.45
37	59.7	106.7	0.56	140.4	173.7	0.81
38	70	92.8	0.75	154.6	213.5	0.72
39	66.8	79.7	0.84	59.2	220.8	0.27
40	161	115	1.4	149.8	152.6	0.98
41	93.7	113.5	0.83	141.1	225.7	0.63
42	133.5	151	0.88	128	203.8	0.63
43	66.7	92.7	0.72	107.4	321.6	0.33
44	146.7	95.7	1.5	133.1	158.6	0.84
45	130.3	122.7	1.1	160.9	289.3	0.56
46	132.7	95.7	1.4	231.1	191	1.2
47	153.7	91.5	1.7	173.4	241.3	0.72
48	75.5	87.7	0.86	123.6	224.9	0.55
1	54	107.7	0.5	87.1	124.9	0.7
2						
3	148.3	175.8	0.84	165.1	158	1
4						
5	113.3	120	0.94	195.4	146.5	1.3
6	88.7	131.3	0.68	130	284.3	0.46
7	138			120		
8	69.7	104.7	0.67	143.1	209.3	0.68
9						
10	219.3	177.7	1.2	201	269.7	0.75

2.2) Serum iron markers for the Kalafong patient group and osteoarthritis patient group

For the purpose of this study it is the iron status and derangements of iron metabolism that were investigated in relation to the expression of the H-subunit and L-subunit of ferritin in the bone marrow macrophage and cells of the erythron. In order to determine the iron status the concentrations of various relevant factors relating to iron metabolism were determined in the blood. These included serum iron, transferrin, transferrin saturation, ferritin and the soluble transferrin receptor (Table 4).

2.3) Red blood production for the Kalafong patient group and osteoarthritis patient group

Red blood cell charateristics are useful in evaluating the iron status since the production of red blood cells is directly influenced by the iron status and inflammatory conditions. Red blood cell production is influenced by inflammatory processes as a result of the derangement in iron metabolism but also due to suppression of the bone marrow. The following were measured in relation to red blood cell production, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width and reticulocyte production index (Table 4).

2.4) Cytokines, C-reactive protein, neopterin, pro-hepcidin and caeruloplasmin

The cytokines investigated included interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (Il-1β), interleukin-6 (Il-6), interleukin-12 (Il-12), interleukin-2 (Il-2), interleukin-8 (Il-8), granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-4 (Il-4), interleukin-5 (Il-5), transforming growth factor-β (TGF-β) and interleukin-10 (Il-10) (Table 5).

Table 4. Serum iron markers and red blood cell production for the Kalafong patient group (patients 1-48) and the osteoarthritis patient group (patients 1-10)

Patient	Iron µmol/l	Trans-ferrin g/l	Trans-ferrin saturation %	Ferritin µg/l	Soluble transferrin receptor µg/ml	Trans-ferrin/ log ferritin	Soluble transferrin R/ log ferritin	RCC x 10^{12}/l	Hb g/dl	Hct l/l	MCV fl	MCH pg	MCHC g/dl	RDW %	RPI
1	2.4	1.58	6.8	171	5	0.71	2.24	2.62	5.6	0.17	65.7	21.3	32.4	20.6	0.15
2	33.8	0.6	252.2	1139	15.5	0.2	5.07	1.55	6.3	0.18	116.2	40.9	35.1	22.7	0.19
3	19.8	1.8	45	224	13.8	0.77	5.87	1.17	3.5	0.11	93.7	29.8	31.8	43.8	1.72
4	5.6	0.99	25.2	7756	3.8	0.26	0.98	3.03	11.8	0.35	114	39.1	34.3	14.2	1.27
5	3.3	1.41	10.5	888	18.5	0.48	6.28	3.33	7.9	0.24	71.3	23.7	33.2	27.3	0.24
6	9.6	2.39	17.9	262	23	0.99	9.51	4.4	9.4	0.29	66	21.4	32.4	20.6	0.42
7	10	0.98	45.7	234	10	0.41	4.22	2.85	8.5	0.24	84.7	29.8	35.1	18.2	1.08
8	6	1.42	18.9	780	2.4	0.49	0.83	3.33	10.3	0.3	89.4	31	34.7	15.9	0.36
9	5.4	1.1	20	1657	6.5	0.34	2.02	3.14	9.4	0.27	86.2	29.8	34.6	16.5	
10	1.3	3.06	1.9	3	52	6.41	108.99	1.54	2.6	0.09	60.8	16.8	27.7	23	
11	5.4	1.53	15.8	334	2.4	0.61	0.95	1.23	4.2	0.12	99.1	34.1	34.4	21.8	0.88
12	13.2	2.77	21.3	58.6	6.5	1.57	3.68	5.77	14.9	0.48	82.4	25.9	31.3	15.7	
13	1.8	0.8	9	3975	4	0.22	1.11	1.91	4.7	0.15	76.8	24.6	32	15.8	
14	7.1	0.94	33.8	2096	5.8	0.28	1.75	3.38	9.5	0.29	85.8	28.2	32.9	15.8	
15	4.9	1.53	14.3	581	13.8	0.55	4.99	2.79	9	0.26	94.3	32.2	34.1	30.1	0.1
16	8.9	1.49	26.7	124	10.5	0.71	5.02	2.12	6.6	0.21	97.4	31.4	32.3	25	2.8
17	16.6	1.4	53	241	4.3	0.59	1.81	3.63	10.2	0.32	86.6	28.1	32.4	14.2	
18	13.4	1.64	36.5	304	32.5	0.66	13.09	0.87	3.2	0.1	113.1	37.1	32.8	20.1	2.04
19	1.7	1.17	6.5	669	9	0.41	3.19	1.96	4.9	0.16	83	25.2	30.4	20.3	0.26
20	2.3	2.15	4.8	83.1	20	1.12	10.42	3.84	9	0.31	79.8	23.5	29.5	18.4	
21	16.4	0.7		7316	8	0.18	2.07	2.7	6.9	0.22	82.3	25.7	31.3	19.8	
22	9.5	0.65		2855	4.5	0.19	1.3	3.78	13.3	0.4	106.5	35.3	33.1	14.2	
23	16.7	1.12	66.5	2016	5	0.34	1.51	2.67	6.9	0.23	84.5	25.7	30.4	19.1	
24	3.3	2.02	7.3	17.6	18	1.62	14.45	2.26	4	0.14	59.6	17.8	29.8	19.2	
25	2.9	3.23	4	3.7	23	5.69	40.48	3.77	5.8	0.21	54.4	15.4	28.4	22.8	0.24
26	10.2	2.3	19.8	116	4	1.11	1.94	4.16	13.7	0.42	100.1	32.9	32.9	14.2	
27	6	2.72	9.9	11	11	2.61	10.56	3.76	10	0.32	84.2	26.5	31.5	15.4	0.59
28	15.6	1.12	62.2	87.3	6	0.58	3.09	2.43	10.7	0.31	125.3	44.1	35.2	15.7	
29	11.4			5672	10.8		2.88	2.2	8.1	0.22	100.2	36.8	36.7	21.3	
30	14.4	2.3	25	50	5.3	1.35	3.12	4.23	13.7	0.435	102.8	32.4	31.5	15.7	
31	7.8	0.6	49	19644	3.5	0.14	0.82	2	4.6	0.142	71	23	32.4	18.7	
32	13.1	3.3	16	15	6.5	2.81	5.53	5.3	14.1	0.47	88.7	26.6	30	14.1	
33	7.4	1	29	45519	26.3	0.22	5.65	1.99	4.1	0.134	67.3	20.6	30.6	30.7	
34	4.8	1.1	18	913	10	0.37	3.38	1.29	3.5	0.129	100	27.1	27.1	18.9	
35	10.5	2.3	18	32	5.3	1.53	3.52	4.18	11.6	0.359	85.9	27.8	32.3	16.9	
36	8.7	0.9	39	2073	5.8	0.27	1.75	1.03	3.7	0.115	111.7	35.9	32.2	24.1	
37	16.9	1.9	35	29.1	11	1.3	7.51	1.66	4	0.132	79.5	24.1	30.3	29	0.08
38	39.6	1.8	88	63.1	14	1	7.78	0.99	2.7	0.087	87.9	27.3	31	33.6	0.06
39	36.6	1.6	91	782.8	4.5	0.55	1.56	0.95	3.3	0.101	106.3	34.7	32.7		0.01
40	8.9	1.4	25	5760	9.5	0.37	2.53	2.08	5.2	0.17	81.7	25	30.6	18	
41	3.9	4.3	4	4	33.5	7.14	55.64	1.57	2.8	0.107	68.2	17.8	26.2	27.1	0.15
42	4	2.9	6	37	36.3	1.85	23.15	2.71	5.9	0.23	84.9	21.8	25.7	33.9	0.63
43	3.3	3.2	4	23.4	25	2.34	18.26	2.99	5.9	0.207	69.2	19.7	28.5	18.5	
44	4.8	1.8	11	289.1	3	0.73	1.22	3.77	9.5	0.301	79.8	25.2	31.6	15.4	
45	13.5	1.5	36	859.8	6.3	0.51	2.15	2.99	9.6	0.305	102	32.1	31.5	14.9	
46	4.5	1.9	9	301.1	7	0.77	2.82	4.69	13.7	0.417	88.9	29.2	32.9	14.4	
47	1.7	1.4	5	266.9	17	0.58	7.01	1.16	3.2	0.101	87.1	27.6	31.7	33.6	0.73
48	6.9	3.4	8	21.6	25	2.55	18.73	2.47	4.5	0.168	68	18.2	26.8	26.7	0.11
1	7.3	2.41	13.5	30.8	4.8	1.62	3.23	4.06	13.5	0.41	101.2	33.3	32.9	12.9	
2	18.6	1.95	42.7	120	1.8	0.94	0.87	4.22	14	0.41	97.4	33.2	34	12.7	
3	14.7	1.68	39.1	219	1.5	0.72	0.64		14.9	0.44					
4	8.8	2.2	17.9	170	3.4	0.99	1.52	4.62	16.1	0.481	104.2	35	33.6	12.4	
5	15	2	33.5	59	3.8	1.13	2.15	5.17	16.1	0.46	88.8	31.2	35.1	12.8	
6	11	2.1	23.4	35	3.4	1.36	2.2	3.91	11.8	0.359	91.9	30.2	32.9	12.6	
7	5.2	0.9	25.9	54	2.5	0.52	1.44	5.2	15.1	0.49	94.3	29.2	30.9	14.1	
8	18.3	1.5	54.5	163	1.8	0.68	0.81	6	17.2	0.535	89.2	28.7	32.2	13.8	
9	7	3	10.4	16	5.8	2.49	4.82	5.04	14.7	0.444	88.1	29.1	33	13.1	
10	22.6	2.4	42.1	75	4	1.28	2.13	5.06	15.2	0.467	92.3	30.1	32.6	12.1	

Table 5. Humoral factors for the Kalafong patient group (patients 1-48) and the osteoarthritis patient group (patients 1-10)

Patient	Il-1	Il-2	Il-4	Il-5	Il-6	Il-8	Il-10	Il-12	TNF-alpha	TGF-beta	INF-gamma	GM-CSF	Neopterin	C-reactive protein	Pro-hepcidin	Caerulo-plasmin
	pg/ml								pg/ml	ng/ml	pg/ml	pg/ml	ng/ml	mg/l	ng/ml	g/l
1	7.4	7.7	3.1	1.7	37.4	115	8.3	13.6	4.8	15.5	0.1	16.5	46.8	137.6	88	0.6
2	2.2	16.3	0.5	1	13	21.2	3	5.8	3.1	3.4	0.1	0.5	15.4	16.7	123	0.3
3	23.8	10.5	2.8	2.6	9739	1020	117	15.2	7	24.5	6.5	33.5	50.1	67	98	0.7
4	0.2	17.5	3.2	7.2	151	37.6	8.3	0.5	2.5	9.4	9.5	0.5	21.3	330	198	0.5
5	30.9	21.4	3.8	2.4	141	68.9	10.1	8.4	15.6	11.8	16	32.1	23.9	118	255	0.4
6	3	8	6.5	1.3	0.5	0.5	10.1	0.5	1.4	4.4	0.1	0.5	1.6	0.33	275	0.4
7	0.2	16.4	2.6	2.4	26.1	19.3	4	8.4	3.3	6.8	1.5	0.5	6.7	28.9	158	0.4
8	12	15.8	5	2.9	8917	1135	11.1	4.1	2.9	3.6	1.5	2	3.4	100	115	0.5
9	3.4	8	5.4	1.6	26.4	77	1.2	0.5	1.3	15.4	0.1	4	6.2	47	108	0.4
10	4	28.3	3.1	2.9	16.3	17	8.7	13.3	5	3	0.1	0.5	4	2.2	140	0.5
11	1.9	13.9	0.5	4.1	44	51.7	4.3	0.5	1.8	3.6	0.1	0.5	66.3	71	250	0.4
12	0.2	7	3.1	1.9	1	11.5	4.3	0.5	3	8	0.1	0.5	2.2	2.5	173	0.7
13	2.6	2.1	5.1	2.6	149	36.7	46	6.2	3.2	5.4	0.5	0.5	37.7	92.4	78	0.4
14	0.2	11.5	2.3	3.1	63.4	39.9	2.4	0.5	3.1	8.3	71.5	8.5	23	138.1	112	0.3
15	0.2	17.6	0.5	2.7	2.4	16.5	3.3	3.5	3	5.4	9.5	240	1.9	0.28	173	0.4
16	0.2	10.1	5.3	3.7	4.7	12.8	11.7	0.5	1.4	17.9	0.1	39.2	10.8	6.3	114	0.4
17	0.2	15.5	2.4	15.5	8.2	39.4	8.2	0.5	2.8	9.1	7	28	52.6	2.5	133	0.4
18	18.6	8.8	0.5	1.8	12.8	18.6	8.7	7.9	8.3	8.3	2.5	25.2	19.4	20.7	149	0.8
19	0.9	21	0.5	2.8	25.5	123	53.9	2.5	4.4	13	7.5	0.5	41.2	28.2	105	
20	0.2	0.5	3.4	0.7	341	27.8	2.7	0.5	0.98	24.4	0.1	2	2.2	90	63	0.4
21	0.2	19.1	3.6	2.9	92	292	5.3	0.5	5.2	8	7.5	5	39	133.1	75	0.5
22	1.2	14.6	0.5	3.4	490	342	15.8	2.7	4.2	8.4	0.1	0.5	38.6	254	149	0.5
23	4.8	11.9	0.5	2.2	5.6	52.8	6.4	0.5	2.1	6.9	2.5	4	20	71	1525	0.4
24	0.2	12.9	4.3	3.6	56.9	26.3	10.7	0.5	1.4	5.5	0.1	47.2	3.9	8.2	154	0.4
25	0.2	4.1	0.5	2	3.6	18.5	4.5	0.5	0.96	13.3	0.1	0.5	4.3	0.65	123	0.4
26	0.2	2.4	0.5	1.2	5.8	19.2	3.5	8.3	3.5	24.7	8.5	7	2	10.1	158	0.3
27	0.96	5.3	0.5	3.7	2.8	15.1	4.4	2.3	3.1	3.9	0.1	15	4.2	1	145	0.3
28	5.4	13	1.8	0.1	46.2	30.4	3.4	3.1	2.3	14	1.5	7	2.7	21.7	122	0.4
29	4.7	4.3	2.2	2.5	155	196	66.7	9.3	4.1	9.3	156	1	80.2	134	102	0.7
30	1.6	15.9	0.5	4.5	2.8	12.9	3.7	7.3	3.2	16	0.1	0.5	1.4	19.6	212	0.5
31	41.2	8.9	5.1	4.1	1655	4466	17.5	3.7	3.8	7	17	1.2	83.5	200	118	0.7
32	0.2	10.2	0.5	2.5	1.5	11	2.1	5.5	2.3	10.5	0.1	0.5	2	3	212	0.4
33	0.2	6.8	3.7	2.1	125	688	316	0.5	6.2	11.5	366	5	55.7	19.7	115	0.6
34	0.2	0.5	4.3	3.6	585	20.9	4.9	0.5	2.8				8.9	45.5	149	
35	0.88	8.6	6.7	3.8	1.5	13.6	4.3	8.5	3.2	25.4	0.1	0.5	2	5.2	198	0.6
36	0.2	2.7	2.4	5	51.8	390	66.9	4.7	8.8	7.9	5.5	0.5	63	45.5	112	0.3
37	0.2	15.6	3.5	0.1	7	8.4	6.9	5.4	3	5.2	1.5	10.1	7.3	2	133	0.2
38	0.2	0.5	0.5	3.2	9.3	19.5	7.4	10.2	2.6	6.5	0.1	59	5.9	1	158	0.6
39	2	0.5	0.5	4.1	2.9	16.1	8.8	3.4	2	11.8	0.1	0.5	17.1	7.3	158	0.9
40	18.5	0.5	5.7	4.5	453	173	10.5	3.6	3.1	17.5	4.5	1.1	38	52.5	180	0.4
41	9.8	0.5	0.5	0.1	1.9	13.3	2.2	7.9	3.7	6.4	0.1	0.5	2.8	0.5	173	0.6
42	1.6	22	2.4	1	5.4	24.1	2.7	6.8	2.2	23.9	0.1	0.5	2.2	0.94	175	0.3
43	0.2	0.5	0.5	3.8	0.89	15.7	2.6	5	1.7	23	0.1	0.5	7.3	1.3	140	0.5
44	1.6	13.6	2	3.8	17.5	108	6.1	4.1	2.2	23.7	0.1	0.5	6.6	20.8	120	0.3
45	0.2	19.7	4.6	19.9	6.9	95.2	1.6	6.6	1.9	9.4	0.1	0.5	12.9	8.6	135	0.3
46	3.1	14.6	0.5	3.6	6.6	12.6	3.5	6.3	4	27.9	0.1	10.1	3.1	16.6	152	0.7
47	1.8	8.9	0.5	2.1	42.8	107	3	4.7	1.8	10.7	29.5	0.5	50.6	53	122	0.4
48	0.2	0.5	0.5	1.5	2.6	6.8	1.4	7.6	2.2	6.5	0.1	1.2	1.9	0.04	114	0.4
1	1.9	10.5	4.8	4.6	7.1	31.3	4.4	2.5	3.7	24.1	0.1	0.5	1.9	5.5	130	0.5
2	10.7	0.5	0.5	2.3	4.7	21.6	2.3	0.5	3.6	18.4	0.1	0.5	2.3	0.56	146	0.5
3	0.2	7.1	0.5	3.5	2.7	11.8	3.1	0.5	3.2	10.4	0.1	0.5	1.7	2.77	122	0.2
4	0.2	14	5.5	3.6	7.6	22.2	2.9	0.5	2.7	23.2	0.1	1.2	3.4	17.3	114	0.5
5	0.2	0.5	0.5	3.7	5.5	16	2	0.5	2.7	18.7	0.1	0.5	1.8	1.59	138	0.6
6	1.7	2	0.5	3.1	3.3	20.4	1.1	0.5	1.6	16.3	0.1	20.5	2.6	2.22	114	0.5
7	0.2	11	3	1.1	8.3	10.8	5.9	0.5	0.92	14.3	0.1	5.8	2	0.26	120	0.3
8	0.2	7.7	5.1	0.1	4.8	20.7	2.7	0.5	2	17.7	0.1	3	1.7	1.22	152	0.5
9	0.2	10.6	4.2	1.5	3.4	23.1	2.3	4.3	3.4	20.9	0.1	0.5	2.1	0.68	155	0.6
10	0.2	3	0.5	0.1	2.3	11.8	0.5	0.5	1.6	18.8	0.1	2.5	2.4	2.04	215	0.3

3) Statistical analysis of the study

For the Kalafong patient group three different subdivisions were investigated statistically. The osteoarthritis patients were treated as a separate group and included with each of the subdivisions of the Kalafong patients for statistical evaluation.

1) Kalafong patients were subdivided into two groups based on normal and elevated C-reactive protein levels. Refer to table 6, table 9 and bar diagram figures 1a-33a.

2) Kalafong patients were subdivided into two groups based on normal and elevated neopterin levels. Refer to table 7, table 10 and bar diagram figures 1b-33b.

3) Kalafong patients were subdivided into two groups based on the presence or absence of an iron transfer block. Refer to table 8, table 11 and bar diagram figures 1c-33c.

For the subdivision based on C-reactive protein and the osteoarthritis patients, the subdivision based on neopterin and the osteoarthritis patients and the subdivision based on the presence or absence of an iron transfer block and the osteoarthritis patients the groups were compared using the Welch t-test and since groups were relatively small and variances could be large, use was also made of the ranksum (Mann-whitney) test. The p-values for both tests were reported and when interpreted, preference was given to the p-value of the ranksum test when the Welch t-test was not significant. Testing was done at the 0.05 level of significance. A p-value of 0.05 and less was taken as statistically significant and a p-value of > 0.05 and < 0.1 was considered as marginally different.

Correlations for the study

Pearson's product-moment correlation coefficient (r) was employed to assess dependence between study parameters.

Table 6. Descriptive statistics and statistical evaluation of expression of the H-subunit and L-subunit of ferritin, serum iron markers, red blood cell production, pro-hepcidin and caeruloplasmin for the C-reactive protein subdivision of the Kalafong patients and the osteoarthritis patients

				CRP elevated vs CRP normal			CRP elevated vs osteoarthritis		CRP normal vs osteoarthritis	
Variable		CRP Elevated	CRP normal	t-test	Mann-Whitney	Osteo-arthritis	t-test	Mann-Whitney	t-test	Mann-Whitney
H-subunit macrophages count/µm²	Mean SD N	96.9 40.6 25	76.5 30 19	0.074	0.159	118.8 56.1 7	0.256	0.374	0.02	0.06
L-subunit macrophages count/µm²	Mean SD N	117.8 45.9 26	107.7 26.8 19	0.397	0.646	136.2 32.8 6	0.363	0.218	0.042	0.098
H-subunit/L-subunit macrophages	Mean SD N	0.99 0.58 25	0.73 0.29 19	0.086	0.205	0.81 0.26 6	0.474	0.549	0.564	0.408
H-subunit cells of erythron count/µm²	Mean SD N	144.4 57.6 25	128.1 48.7 19	0.326	0.456	148.8 41.2 7	0.853	0.538	0.329	0.193
L-subunit cells of erythron count/µm²	Mean SD N	212.3 76.6 26	215.6 54.4 19	0.873	0.581	198.8 66.8 6	0.693	0.809	0.537	0.525
H-subunit/L-subunit cells of erythron	Mean SD N	0.78 0.47 25	0.61 0.24 19	0.163	0.414	0.83 0.31 6	0.831	0.549	0.091	0.075
Serum iron µmol/l	Mean SD N	9 6.9 29	11.3 10.6 19	0.405	0.898	12.9 5.9 10	0.102	0.058	0.619	0.142
Transferrin g/l	Mean SD N	1.3 0.5 28	2.5 0.83 19	0	0.0002	2 0.57 10	0.003	0.002	0.098	0.261
Transferrin saturation %	Mean SD N	34.2 47.7 26	24.3 26.5 19	0.382	0.106	30.3 14.4 10	0.71	0.437	0.439	0.094
Ferritin µg/l	Mean SD N	3903.1 8942.6 29	166.8 270.3 19	0.033	0.002	94.2 69.6 10	0.03	0.0001	0.281	0.359
Soluble transferrin receptor µg/ml	Mean SD N	9.5 7.4 29	17.3 12.9 19	0.023	0.336	3.3 1.4 10	0.0001	0.0006	0.0002	0

Transferrin/ log ferritin	Mean SD N	0.51 0.31 28	2.2 2 19	0.002	0.0008	1.2 0.58 10	0.005	0.0003	0.044	0.183
Soluble transferrin receptor/ log ferritin	Mean SD N	3.4 2.9 29	18.1 26.1 19	0.025	0.065	2 1.3 10	0.04	0.131	0.015	0.0003
Red blood cell count x 10^{12}/l	Mean SD N	2.6 1.1 29	2.9 1.4 19	0.322	0.0008	4.8 0.67 9	0	0	0	0.002
Haemoglobin g/dl	Mean SD N	7.6 3.5 29	7.2 3.8 19	0.739	0.004	14.9 1.5 10	0	0	0	0.0001
Haematocrit l/l	Mean SD N	0.23 0.1 29	0.24 0.12 19	0.908	0.001	0.45 0.05 10	0	0	0	0.0002
Mean corpuscular volume fl	Mean SD N	91.3 15.6 29	80.3 14.9 19	0.018	0.926	94.2 5.7 9	0.416	0.3	0.002	0.006
Mean corpuscular haemoglobin pg	Mean SD N	29.8 6.1 29	24.5 5.9 19	0.004	0.71	31.1 2.2 9	0.344	0.287	0.0002	0.003
Mean corpuscular haemoglobin concentration g/dl	Mean SD N	32.5 2 29	30.3 2.4 19	0.002	0.353	33 1.2 9	0.373	0.39	0.0003	0.002
Red blood cell distribution width %	Mean SD N	20.2 6.6 29	21.1 8.2 19	0.689	0.479	12.9 0.64 9	0	0	0.0004	0.0002
RPI	Mean SD N	0.81 0.65 11	0.47 0.8 11	0.29	0.039					
Neopterin ng/ml	Mean SD N	29.5 24.8 29	7.7 11.7 19	0.0002	0	2.2 0.52 10	0	0.0001	0.053	0.017
Pro-hepcidin ng/ml	Mean SD N	135.2 48.2 28	159.3 38.6 19	0.065	0.039	140.6 30.2 10	0.686	0.479	0.164	0.118
Caeruloplasmin g/l	Mean SD N	0.47 0.15 27	0.46 0.16 19	0.733	0.643	0.45 0.14 10	0.644	0.986	0.891	0.76
CD4 x 10^{6}/l	Mean SD N	293 397.7 20	449.3 664.9 10	0.505	0.072					

Table 7. Descriptive statistics and statistical evaluation of expression of the H-subunit and L-subunit of ferritin, serum iron markers, red blood cell production, pro-hepcidin and caeruloplasmin for the neopterin subdivision of the Kalafong patients and the osteoarthritis patients

				Neopterin elevated vs neopterin normal			Neopterin elevated vs osteoarthritis		Neopterin normal vs osteoarthritis	
Variable		**Neopterin elevated**	**Neopterin normal**	**t-test**	**Mann-Whitney**	**Osteo-arthritis**	**t-test**	**Mann-Whitney**	**t-test**	**Mann-Whitney**
H-subunit macrophages count/µm²	Mean SD N	94.7 37.3 31	72.4 34 13	0.071	0.037	118.8 56.1 7	0.17	0.3	0.033	0.052
L-subunit macrophages count/µm²	Mean SD N	108.1 34.3 32	126.8 47.5 13	0.147	0.26	136.2 32.8 6	0.073	0.085	0.668	0.456
H-subunit/L-subunit macrophages	Mean SD N	0.97 0.49 31	0.66 0.43 13	0.051	0.023	0.81 0.26 6	0.445	0.564	0.43	0.188
H-subunit cells of erythron count/µm²	Mean SD N	141.4 54.6 31	127.8 53.3 13	0.452	0.322	148.8 41.2 7	0.739	0.463	0.378	0.191
L-subunit cells of erythron count/µm²	Mean SD N	200.7 54.9 31	245.7 85.7 13	0.41	0.115	198.8 66.8 6	0.938	0.873	0.255	0.335
H-subunit/L-subunit cells of erythron	Mean SD N	0.77 0.42 31	0.57 0.31 13	0.139	0.062	0.83 0.31 6	0.742	0.51	0.116	0.035
Serum iron µmol/l	Mean SD N	10.4 9.6 35	8.7 4.5 13	0.423	0.898	12.9 5.9 10	0.322	0.096	0.08	0.063
Transferrin g/l	Mean SD N	1.5 0.7 34	2.5 0.84 13	0.0008	0.0002	2 0.57 10	0.025	0.012	0.104	0.153
Transferrin saturation %	Mean SD N	35.2 45.8 32	17.4 15.1 13	0.058	0.106	30.3 14.4 10	0.604	0.497	0.049	0.028
Ferritin µg/l	Mean SD	3277.4 8236	126.8 164.5	0.03	0.002	94.2 69.6	0.029	0.003	0.528	0.804

	N	35	13			10				
Soluble transferrin receptor µg/ml	Mean SD N	11.8 10.2 35	14.8 11.5 13	0.416	0.336	3.3 1.4 10	0	0.0002	0.004	0.0002
Transferrin/ log ferritin	Mean SD N	0.96 1.4 34	1.8 1.7 13	0.118	0.0008	1.2 0.57 10	0.48	0.008	0.213	0.306
Soluble transferrin receptor/ log ferritin	Mean SD N	8.5 19 35	11.2 14.9 13	0.599	0.065	2 1.3 10	0.053	0.042	0.045	0.001
Red blood cell count x 10^{12}/l	Mean SD N	2.3 0.95 35	3.7 1.2 13	0.002	0.0008	4.8 0.67 9	0	0	0.161	0.057
Haemoglobin g/dl	Mean SD N	6.4 2.9 35	10.2 3.9 13	0.005	0.004	14.9 1.5 10	0	0	0.001	0.001
Haematocrit l/l	Mean SD N	0.2 0.08 35	0.33 0.12 13	0.002	0.001	0.45 0.05 10	0	0	0.003	0.01
Mean corpuscular volume fl	Mean SD N	86.8 16.2 35	87.3 16.3 13	0.925	0.926	94.2 5.7 9	0.034	0.081	0.182	0.082
Mean corpuscular haemoglobin pg	Mean SD N	27.9 6.3 35	27.2 7.2 13	0.767	0.71	31.1 2.2 9	0.018	0.074	0.087	0.053
Mean corpuscular haemoglobin concentration g/dl	Mean SD N	31.9 2.2 35	30.8 3 13	0.247	0.353	33 1.2 9	0.051	0.081	0.03	0.066
Red blood cell distribution width %	Mean SD N	20.6 7.5 35	20.3 6.8 13	0.872	0.479	12.9 0.64 9	0	0	0.002	0.0001
RPI	Mean SD N	0.75 0.8 17	0.28 0.23 5	0.047	0.272					
C-reactive protein mg/l	Mean SD N	64.7 77.2 35	13.1 24.4 13	0.001	0.002	3.4 5.1 10	0	0.0004	0.184	0.457
Pro-hepcidin ng/ml	Mean SD N	135.6 40 34	169.2 52.2 13	0.05	0.02	140.6 30.2 10	0.678	0.642	0.114	0.071
Caeruloplasmin g/l	Mean SD	0.47 0.16	0.47 0.14	0.957	0.889	0.45 0.14	0.748	0.848	0.741	0.975

	N	33	13			10				
CD4 x 10^6/l	Mean SD N	304.6 492.6 27	710 443.1 3	0.229	0.072					

Table 8. Descriptive statistics and statistical evaluation of expression of the H-subunit and L-subunit of ferritin, serum iron markers, red blood cell production, pro-hepcidin and caeruloplasmin for the iron transfer block subdivision of the Kalafong patients and the osteoarthritis patients

				Fe-block vs no Fe-block			Fe-block vs osteoarthritis		No Fe-block vs Osteoarthritis	
Variable		**Fe-block**	**No Fe-block**	**t-test**	**Mann-Whitney**	**Osteo-arthritis**	**t-test**	**Mann-Whitney**	**t-test**	**Mann-Whitney**
H-subunit macrophages count/µm²	Mean SD N	99.5 41 22	78.5 30.8 23	0.06	0.12	118.8 56.1 7	0.424	0.445	0.109	0.074
L-subunit macrophages count/µm²	Mean SD N	113.1 39.4 21	113.6 40.3 23	0.968	0.981	136.2 32.8 6	0.175	0.18	0.181	0.139
H-subunit/L-subunit macrophages	Mean SD N	1 0.57 21	0.76 0.39 23	0.116	0.178	0.81 0.26 6	0.251	0.448	0.732	0.451
H-subunit cells of erythron count/µm²	Mean SD N	141.5 50.9 22	135.1 57.1 23	0.691	0.364	148.8 41.2 7	0.706	0.508	0.493	0.292
L-subunit cells of erythron count/µm²	Mean SD N	211.5 59.6 21	217.5 76.1 23	0.774	0.972	198.8 66.8 6	0.684	0.641	0.567	0.706
H-subunit/L-subunit cells of erythron	Mean SD N	0.74 0.44 21	0.68 0.36 23	0.651	0.647	0.83 0.31 6	0.588	0.414	0.28	0.161
Serum iron µmol/l	Mean SD N	9.1 7.5 25	10.8 9.6 23	0.514	0.718	12.9 5.9 10	0.132	0.053	0.451	0.137
Transferrin g/l	Mean SD N	1.3 0.42 24	2.3 0.92 23	0	0.0001	2 0.57 10	0.003	0.001	0.328	0.457

Transferrin saturation %	Mean SD N	27.7 21.7 22	32.3 52.5 23	0.702	0.301	30.3 14.4 10	0.69	0.371	0.869	0.153
Ferritin µg/l	Mean SD N	2709.6 4210.9 25	2113.8 9465 23	0.783	0	94.2 69.6 10	0.005	0.0001	0.317	0.814
Soluble transferrin receptor µg/ml	Mean SD N	7.1 4.4 25	18.5 12.1 23	0.0002	0	3.3 1.4 10	0.0004	0.002	0	0
Transferrin/ log ferritin	Mean SD N	0.48 0.28 24	2 1.9 23	0.002	0	1.2 0.57 10	0.004	0.0002	0.088	0.457
Soluble transferrin receptor/ log ferritin	Mean SD N	2.6 1.9 25	16.5 23.9 23	0.011	0	2 1.3 10	0.313	0.454	0.008	0
Red blood cell count x 10^{12}/l	Mean SD N	2.5 0.95 25	2.9 1.4 23	0.237	0.348	4.8 0.67 9	0	0	0	0.002
Haemoglobin g/dl	Mean SD N	7.3 3.2 25	7.5 4.1 23	0.834	0.934	14.9 1.5 10	0	0	0	0
Haematocrit l/l	Mean SD N	0.22 0.09 25	0.24 0.13 23	0.558	0.796	0.45 0.05 10	0	0	0	0.0002
Mean corpuscular volume fl	Mean SD N	89.8 13 25	83.9 18.6 23	0.214	0.208	94.2 5.7 9	0.186	0.212	0.024	0.024
Mean corpuscular haemoglobin pg	Mean SD N	29.1 5 25	26.2 7.7 23	0.122	0.101	31.1 2.2 9	0.12	0.178	0.009	0.019
Mean corpuscular haemoglobin concentration g/dl	Mean SD N	32.4 1.9 25	30.8 2.7 23	0.024	0.027	33 1.2 9	0.263	0.274	0.003	0.013
Red blood cell distribution width %	Mean SD N	19.7 5.5 24	22.3 7.5 23	0.197	0.273	12.9 0.64 9	0	0	0	0
RPI	Mean SD N	0.41 0.41 10	0.84 0.89 12	0.158	0.291					

Neopterin ng/ml	Mean SD N	31.7 24.5 25	9.2 14.5 23	0.0004	0.0002	2.2 0.52 10	0	0	0.03	0.014
C-reactive protein mg/l	Mean SD N	84.1 83.2 25	14.5 22.3 23	0.0004	0.0001	3.4 5.1 10	0.0001	0.0001	0.03	0.147
Pro-hepcidin ng/ml	Mean SD N	194 281 25	151.6 44.7 23	0.463	0.256	140.6 30.2 10	0.357	0.688	0.418	0.367
Caeruloplasmin g/l	Mean SD N	0.44 0.16 23	0.49 0.14 23	0.291	0.214	0.45 0.14 10	0.905	0.507	0.439	0.674
CD4 x 10^6/l	Mean SD N									

Table 9. Descriptive statistics and statistical evaluation of all cytokines for the C-reactive protein subdivision of the Kalafong patients and the osteoarthritis patients

				CRP elevated vs CRP normal			CRP elevated vs osteoarthritis		CRP normal vs osteoarthritis	
Variable		**CRP elevated**	**CRP normal**	**t-test**	**Mann-Whitney**	**Osteo-arthritis**	**t-test**	**Mann-Whitney**	**t-test**	**Mann-Whitney**
GM-CSF pg/ml	Mean SD N	6.1 9.4 28	23.4 55.5 19	0.194	0.882	3.6 6.2 10	0.344	0.448	0.14	0.763
Interleukin-1beta pg/ml	Mean SD N	6.5 10.3 29	1.3 2.3 19	0.013	0.01	1.6 3.3 10	0.029	0.036	0.816	0.829
Interleukin-2 pg/ml	Mean SD N	10.7 6.3 29	9.9 8.2 19	0.72	0.499	6.7 4.9 10	0.051	0.067	0.203	0.488
Interleukin-4 pg/ml	Mean SD N	2.5 1.8 29	2.4 2.2 19	0.917	0.761	2.5 2.2 10	0.996	0.947	0.939	0.942
Interleukin-5 pg/ml	Mean SD N	2.8 1.5 29	4.1 5 19	0.313	0.924	2.4 1.6 10	0.418	0.552	0.189	0.462
Interleukin-6	Mean	807.6	7.2	0.081	0	5	0.081	0	0.468	0.313

pg/ml	SD N	2384.6 29	12.6 19			2.1 10				
Interleukin-8 pg/ml	Mean SD N	334.6 845.2 29	20.1 19.9 19	0.055	0	18.9 6.4 10	0.054	0.0009	0.823	0.359
Interleukin-10 pg/ml	Mean SD N	28.1 61.6 29	5.6 3.3 19	0.059	0.09	2.7 1.5 10	0.035	0.0005	0.004	0.028
Interleukin-12 pg/ml	Mean SD N	4.6 4 29	4.7 3.8 19	0.962	0.906	1.1 1.3 10	0.0001	0.005	0.001	0.007
TNF-alpha pg/ml	Mean SD N	4.1 2.9 29	2.5 0.97 19	0.007	0.007	2.5 0.96 10	0.014	0.067	0.843	0.714
TGF-beta ng/ml	Mean SD N	12.4 7.1 28	10.5 7.1 19	0.369	0.209	18.3 4.1 10	0.003	0.011	0.0007	0.005
INF-gamma pg/ml	Mean SD N	25.6 73.8 28	1 2.6 19	0.09	0.0008	0.1 0 10	0.079	0.0008	0.135	0.193

Table 10. Descriptive statistics and statistical evaluation of all cytokines for the neopterin subdivision of the Kalafong patients and the osteoarthritis patients

				Neopterin elevated vs neopterin normal			Neopterin elevated vs osteoarthritis		Neopterin normal vs osteoarthritis	
Variable		**Neopterin elevated**	**Neopterin normal**	**t-test**	**Mann-Whitney**	**Osteo-arthritis**	**t-test**	**Mann-Whitney**	**t-test**	**Mann-Whitney**
GM-CSF pg/ml	Mean SD N	10.2 15.6 34	20.8 65.9 13	0.574	0.482	3.6 6.2 10	0.054	0.427	0.366	0.947
Interleukin-1beta pg/ml	Mean SD N	5.4 9.7 35	2 2.8 13	0.075	0.537	1.6 3.3 10	0.057	0.117	0.721	0.373
Interleukin-2 pg/ml	Mean SD N	10.7 7.1 35	9.3 7.1 13	0.538	0.577	6.7 4.9 10	0.049	0.101	0.306	0.455
Interleukin-4 pg/ml	Mean SD N	2.6 1.8 35	2.1 2.3 13	0.472	0.271	2.5 2.2 10	0.888	0.866	0.673	0.713

Interleukin-5 pg/ml	Mean SD N	3.9 3.7 35	1.9 1.4 13	0.011	0.011	2.4 1.6 10	0.072	0.219	0.478	0.514
Interleukin-6 pg/ml	Mean SD N	661.1 2187.6 35	32.3 93.6 13	0.099	0.0003	5 2.1 10	0.085	0.0005	0.314	0.193
Interleukin-8 pg/ml	Mean SD N	282.4 775.9 35	15.4 8.3 13	0.05	0.0001	18.9 6.4 10	0.053	0.007	0.258	0.321
Interleukin-10 pg/ml	Mean SD N	24.9 56.3 35	3.6 2.1 13	0.032	0.0006	2.7 1.5 10	0.026	0.0002	0.238	0.193
Interleukin-12 pg/ml	Mean SD N	4.5 4.2 35	5.1 3.1 13	0.587	0.364	1.1 1.3 10	0.0001	0.007	0.0005	0.003
TNF-alpha pg/ml	Mean SD N	3.8 2.8 35	2.7 0.89 13	0.048	0.451	2.5 0.96 10	0.035	0.252	0.722	0.664
TGF-beta ng/ml	Mean SD N	10.2 5.8 34	15.2 8.9 13	0.082	0.119	18.3 4.1 10	0.0001	0.0004	0.281	0.457
INF-gamma pg/ml	Mean SD N	21 67.4 34	1.6 3.3 13	0.103	0.056	0.1 0 10	0.079	0.003	0.135	0.112

Table 11. Descriptive statistics and statistical evaluation of all cytokines for the iron transfer block subdivision of the Kalafong patients and the osteoarthritis patients

				Fe-block vs no Fe-block			Fe-block vs osteoarthritis		No Fe-block vs osteoarthritis	
Variable		Fe-block	No Fe-block	t-test	Mann-Whitney	Osteo-arthritis	t-test	Mann-Whitney	t-test	Mann-Whitney
GM-CSF pg/ml	Mean SD N	15.2 48.6 24	10.9 17.4 23	0.684	0.937	3.6 6.2 10	0.259	0.514	0.085	0.628
Interleukin-1beta pg/ml	Mean SD N	5.5 10.3 25	3.4 6.1 23	0.386	0.497	1.6 3.3 10	0.099	0.107	0.286	0.275
Interleukin-2 pg/ml	Mean SD	11 6.9	9.6 7.3	0.503	0.363	6.7 4.9	0.047	0.062	0.184	0.409

	N	25	23			10				
Interleukin-4 pg/ml	Mean SD N	2.7 1.9 25	2.2 2 23	0.39	0.306	2.5 2.2 10	0.8	0.764	0.734	0.755
Interleukin-5 pg/ml	Mean SD N	4.2 4.3 25	2.3 1.3 23	0.045	0.043	2.4 1.6 10	0.07	0.214	0.957	0.984
Interleukin-6 pg/ml	Mean SD N	525.4 1782.4 25	453.1 2025.5 23	0.896	0.002	5 2.1 10	0.157	0.0005	0.3	0.667
Interleukin-8 pg/ml	Mean SD N	321.1 893.6 25	89.4 246.5 23	0.223	0.0001	18.9 6.4 10	0.104	0.0007	0.185	0.695
Interleukin-10 pg/ml	Mean SD N	15.2 19.9 25	23.5 68 23	0.582	0.122	2.7 1.5 10	0.005	0.0007	0.158	0.014
Interleukin-12 pg/ml	Mean SD N	4 3.4 25	5.4 4.3 23	0.2	0.224	1.1 1.3 10	0.0009	0.008	0.0001	0.004
TNF-alpha pg/ml	Mean SD N	3.8 2.9 25	3.2 1.9 23	0.391	0.477	2.5 0.96 10	0.071	0.177	0.228	0.638
TGF-beta ng/ml	Mean SD N	10.4 5.6 24	12.8 8.3 23	0.258	0.64	18.3 4.1 10	0.0001	0.0007	0.016	0.06
INF-gamma pg/ml	Mean SD N	14.8 33.7 24	16.5 76.2 23	0.924	0.0007	0.1 0 10	0.043	0.0006	0.313	0.117

4) Bar diagrams for variables for the different subdivisions

Figures 1a-33a contain bar diagrams for all variables for the C-reactive protein subdivision. Figures 1b-33b contain bar diagrams for all variables for the neopterin subdivision. Figures 1c-33c contain bar diagrams for all variables for the iron transfer block subdivision. Error bars refer to the standard deviation.

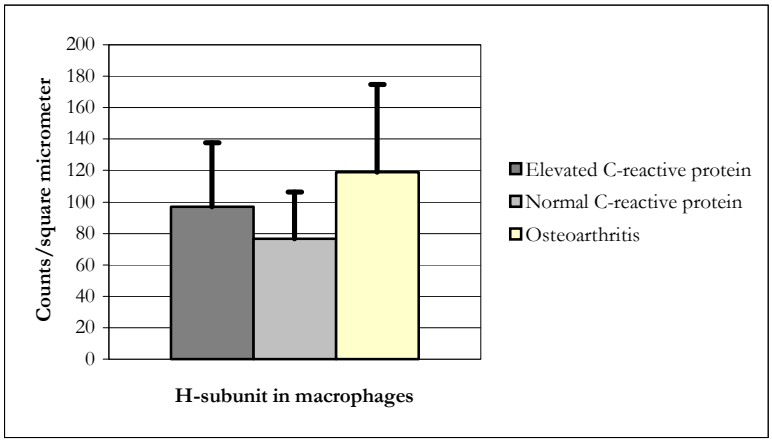

Figure 1a.
Marginally higher for elevated CRP group compared to normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Significantly higher for osteoarthritis group compared to normal CRP group.

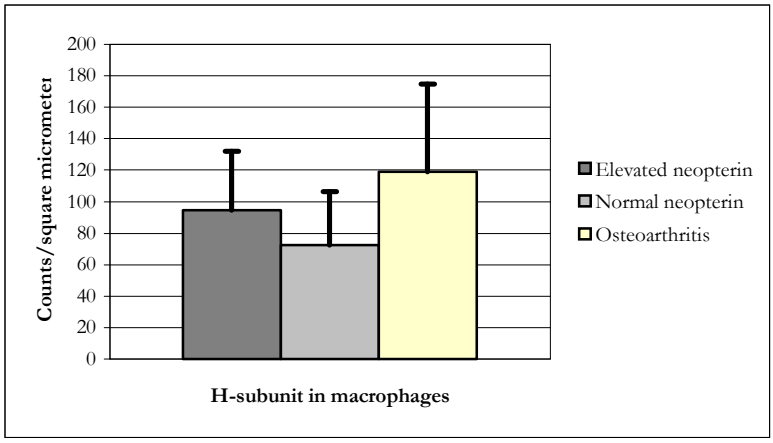

Figure 1b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
Significantly higher for osteoarthritis group compared to normal neopterin group.

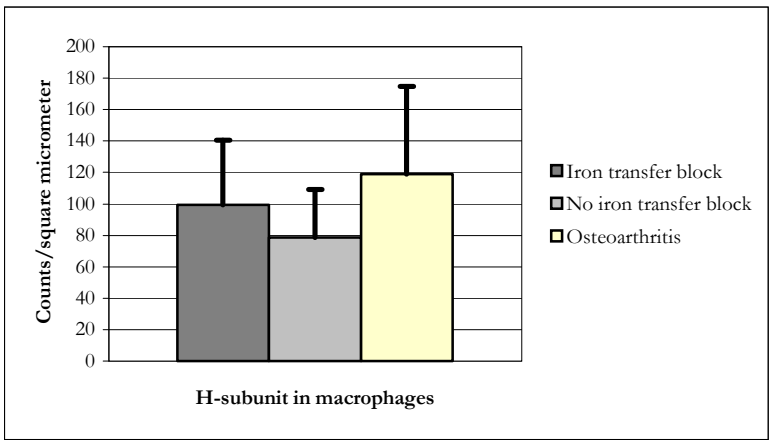

Figure 1c.
Marginally higher for iron transfer block group compared to no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Marginally higher for osteoarthritis group compared to no iron transfer block group.

Figure 1a – 1c. Differences in expression of the H-subunit of ferritin in the macrophage for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

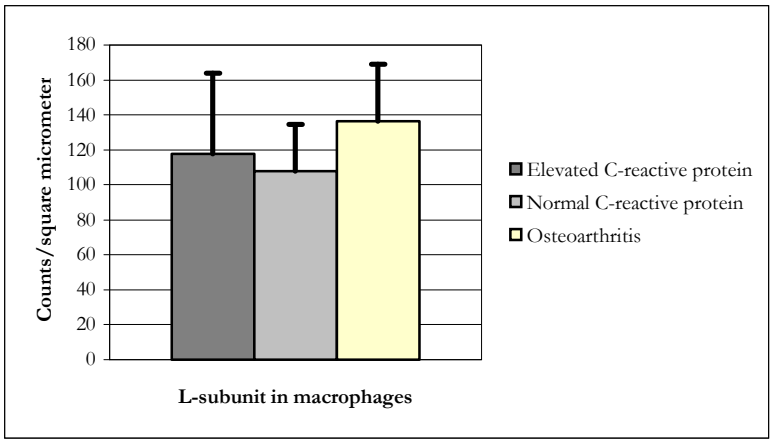

Figure 2a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Significantly higher for osteoarthritis group compared to normal CRP group.

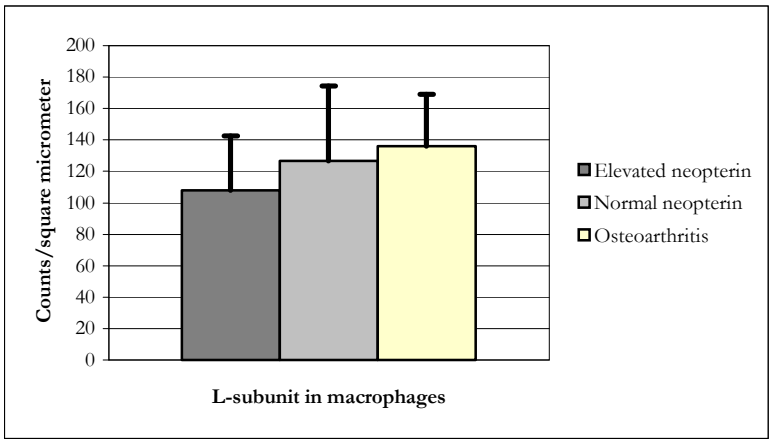

Figure 2b.
No significant difference between elevated neopterin group and normal neopterin group.
Marginally lower for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

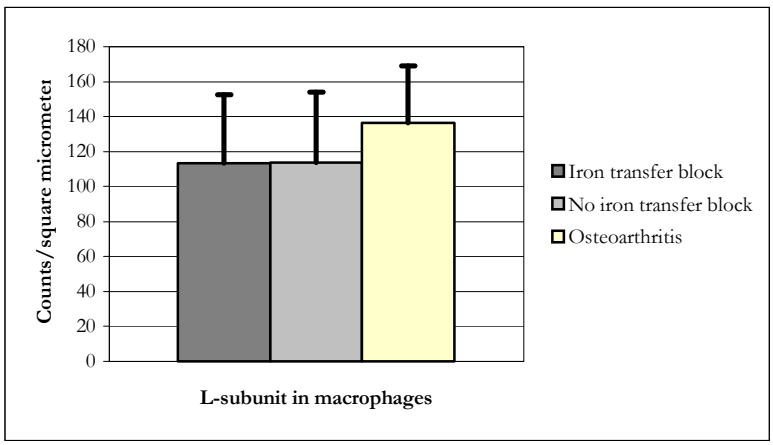

Figure 2c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 2a – 2c. Differences in expression of the L-subunit of ferritin in the macrophage for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

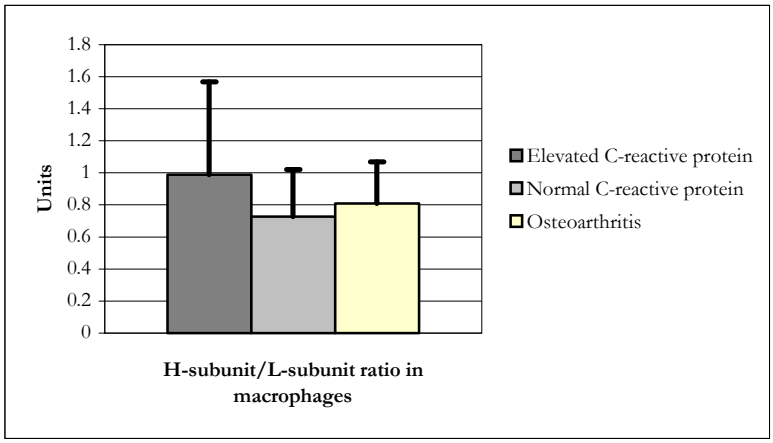

Figure 3a.
Marginally higher for elevated CRP group compared to normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

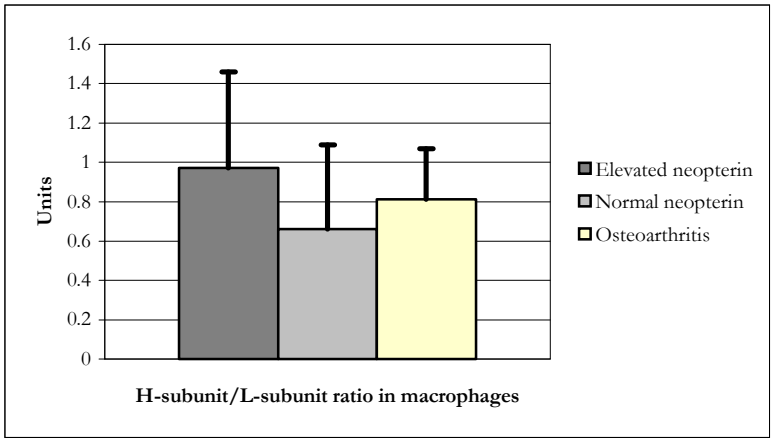

Figure 3b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

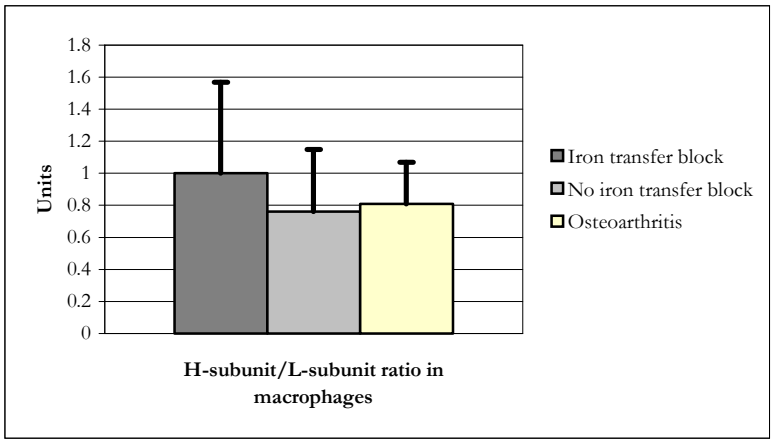

Figure 3c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 3a – 3c. Differences in H-subunit/L-subunit ratio in the macrophage for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

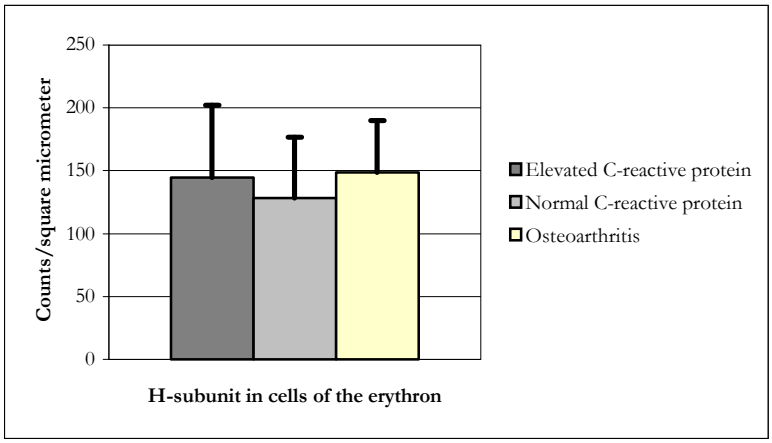

Figure 4a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

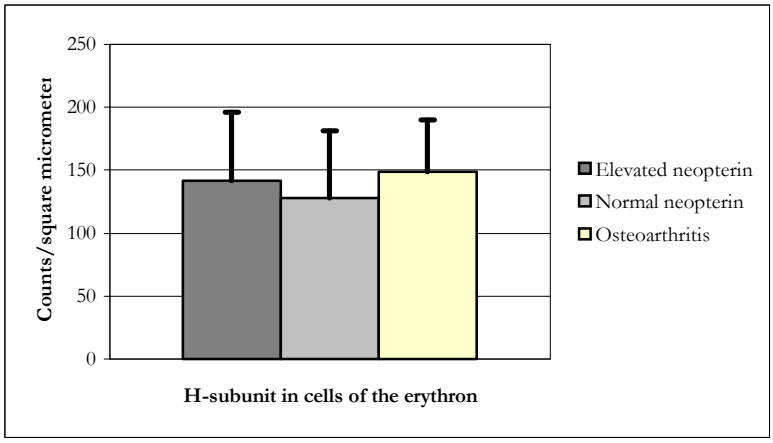

Figure 4b.
No significant difference between elevated neopterin group and normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

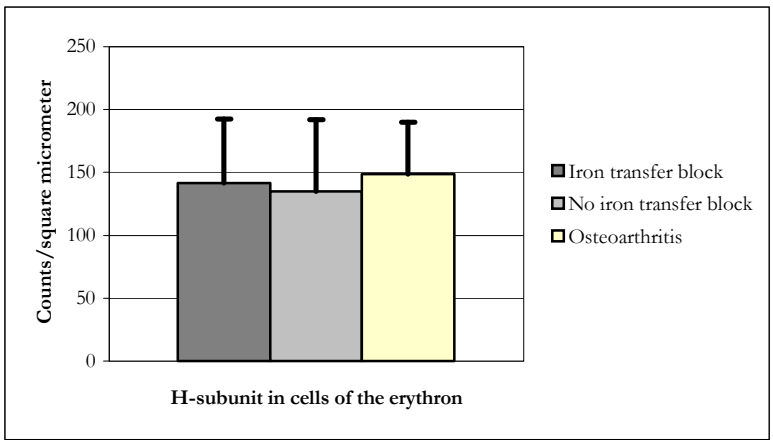

Figure 4c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 4a – 4c. Differences in expression of the H-subunit of ferritin in cells of the erythron for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

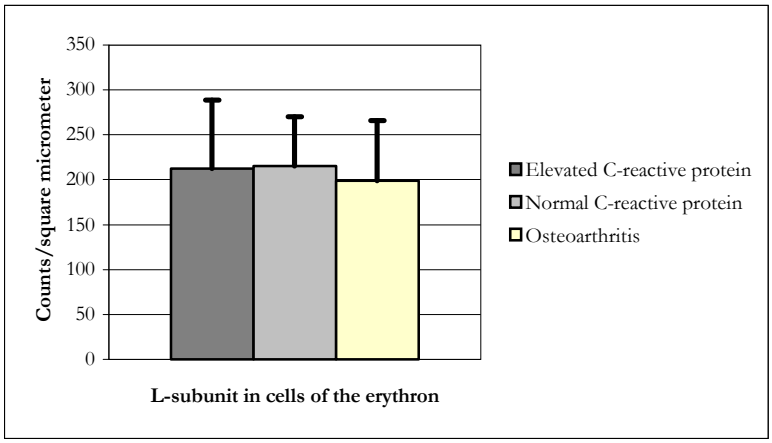

Figure 5a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

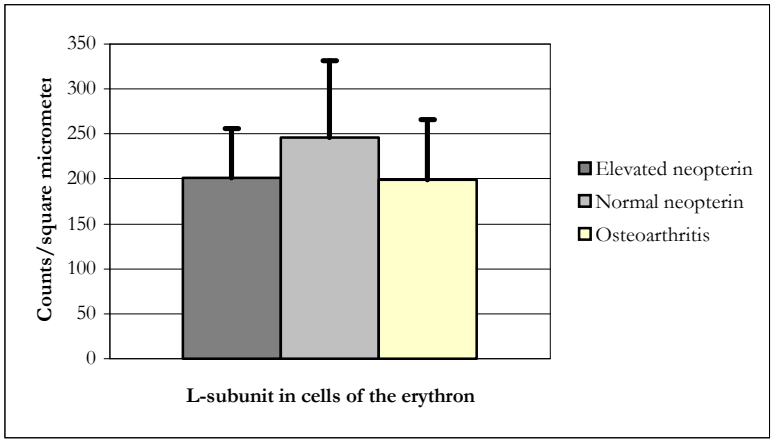

Figure 5b.
No significant difference between elevated neopterin group and normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

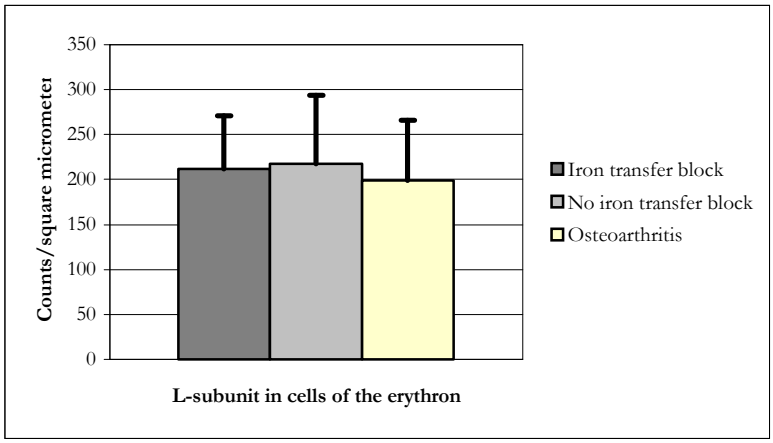

Figure 5c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 5a – 5c. Differences in expression of the L-subunit of ferritin in cells of the erythron for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

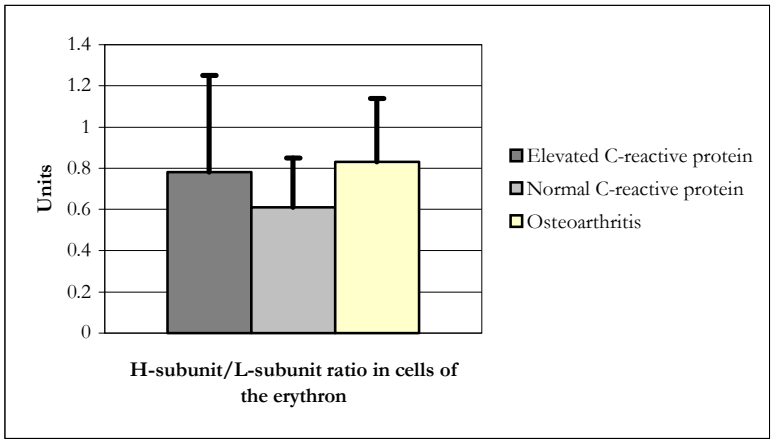

Figure 6a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Marginally higher for osteoarthritis group compared to normal CRP group.

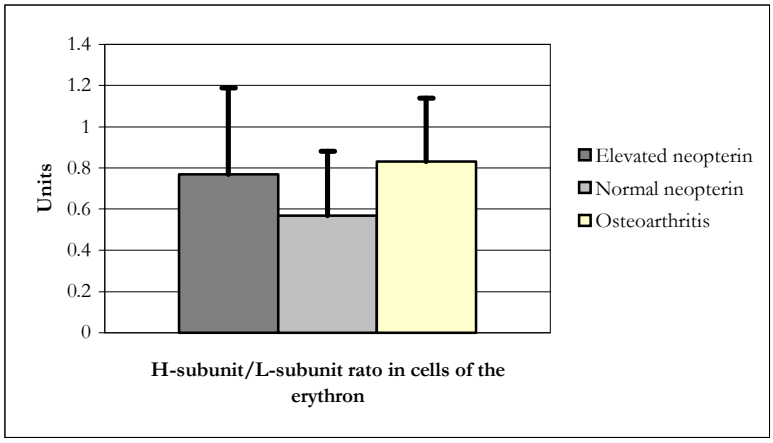

Figure 6b.
Marginally higher for elevated neopterin group compared to normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
Significantly higher for osteoarthritis group compared to normal neopterin group.

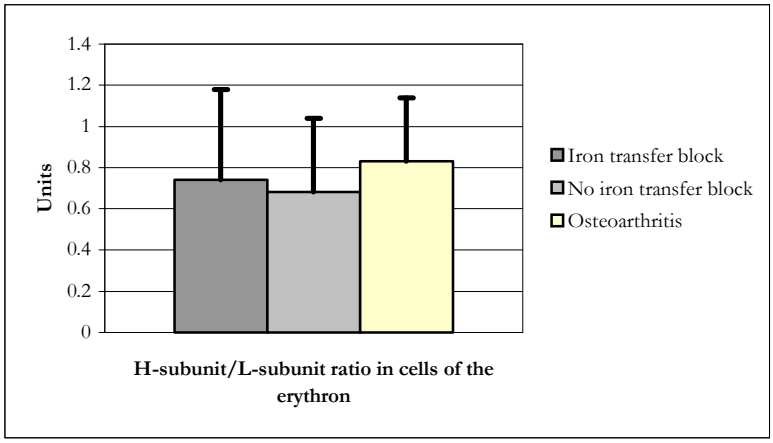

Figure 6c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 6a – 6c. Differences in H-subunit/L-subunit ratio in cells of the erythron for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

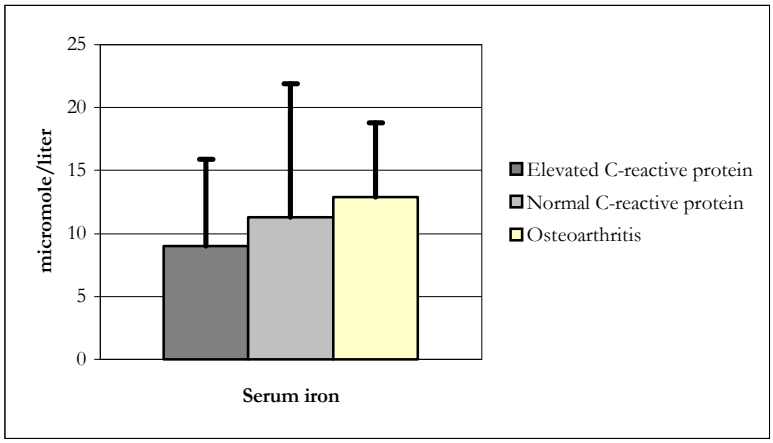

Figure 7a.
No significant difference between elevated CRP group and normal CRP group.
Marginally lower for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

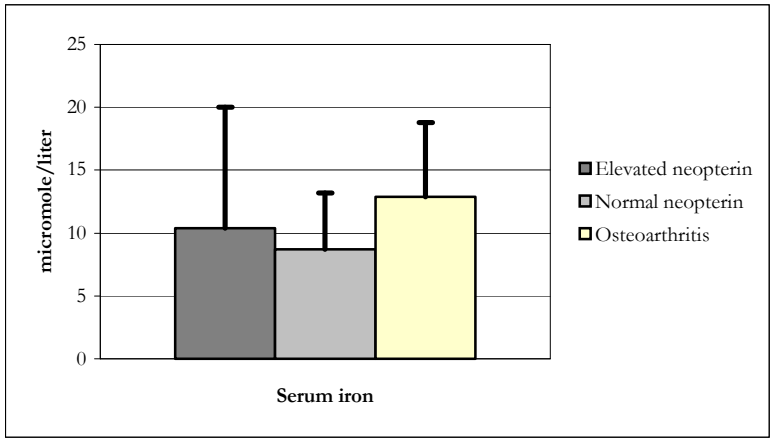

Figure 7b.
No significant difference between elevated neopterin group and normal neopterin group.
Marginally lower for elevated neopterin group compared to osteaoarthritis group.
Marginally lower for normal neopterin group compared to osteoarthritis group.

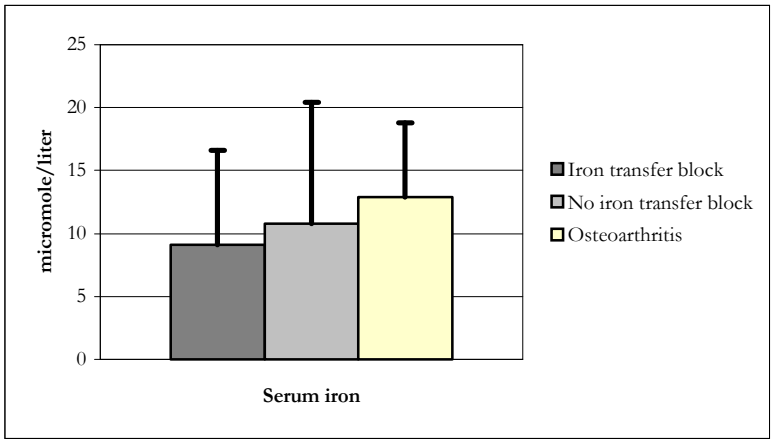

Figure 7c.
No significant difference between iron transfer block group and no iron transfer block group.
Marginally lower for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 7a – 7c. Differences in serum iron for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

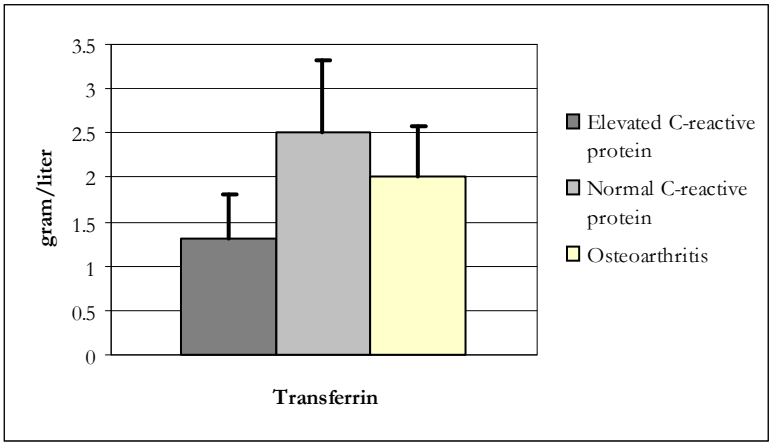

Figure 8a.
Significantly lower for elevated CRP group compared to normal CRP group.
Significantly lower for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

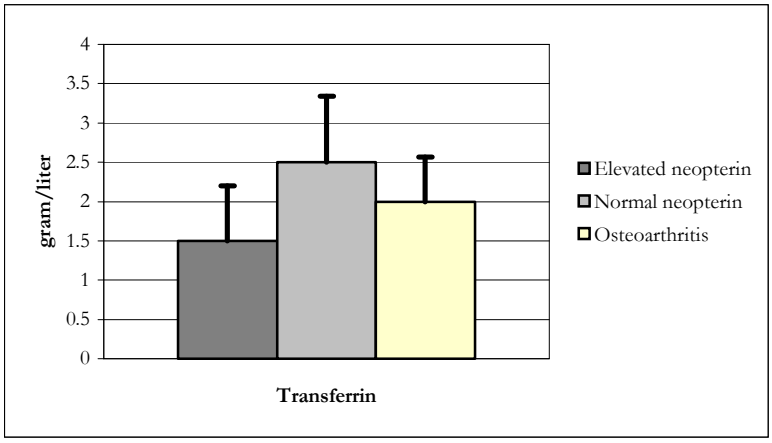

Figure 8b.
Significantly lower for elevated neopterin group compared to normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

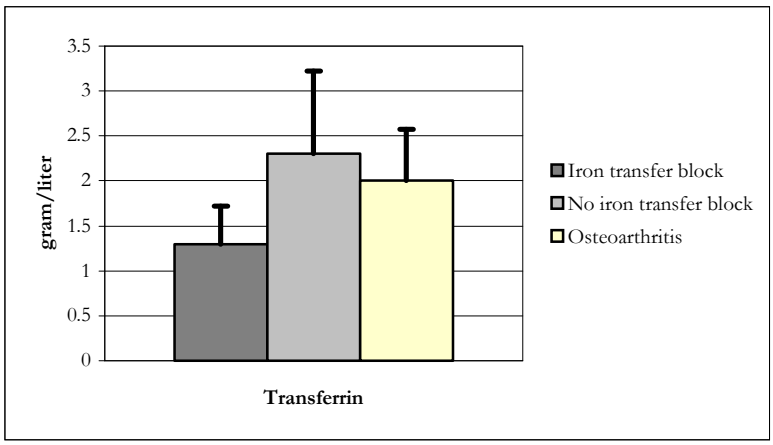

Figure 8c.
Significantly lower for iron transfer block group compared to no iron transfer block group.
Significantly lower for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 8a – 8c. Differences in transferrin for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

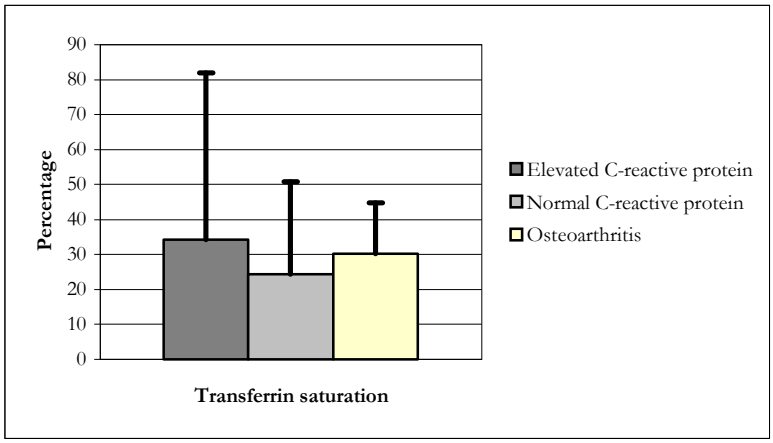

Figure 9a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

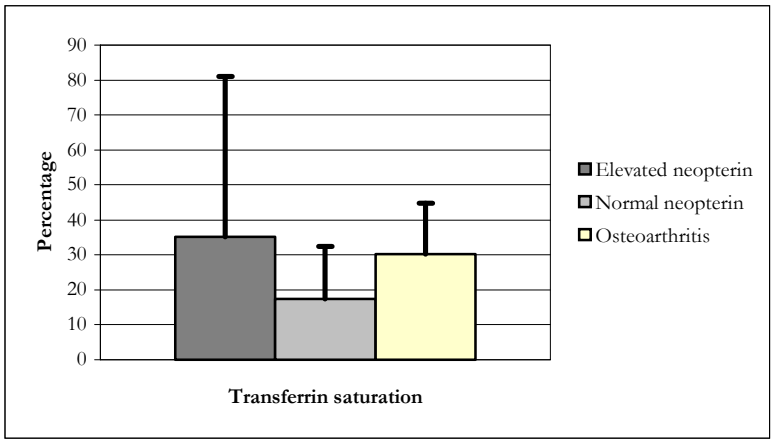

Figure 9b.
Marginally lower for normal neopterin group compared to elevated neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
Significantly lower for normal neopterin group compared to osteoarthritis group.

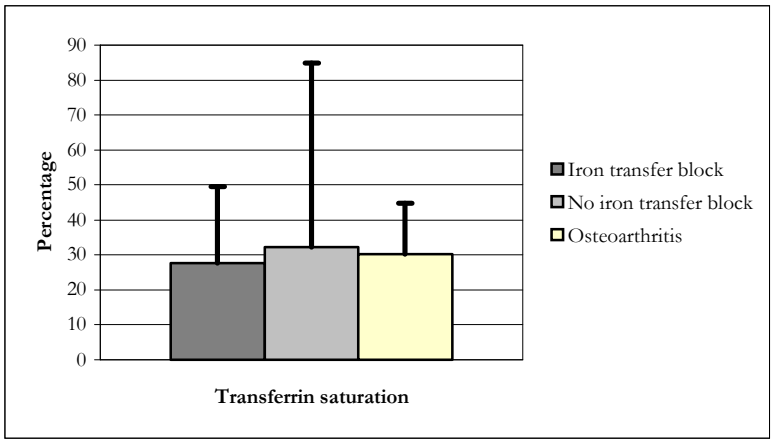

Figure 9c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 9a – 9c. Differences in transferrin saturation for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

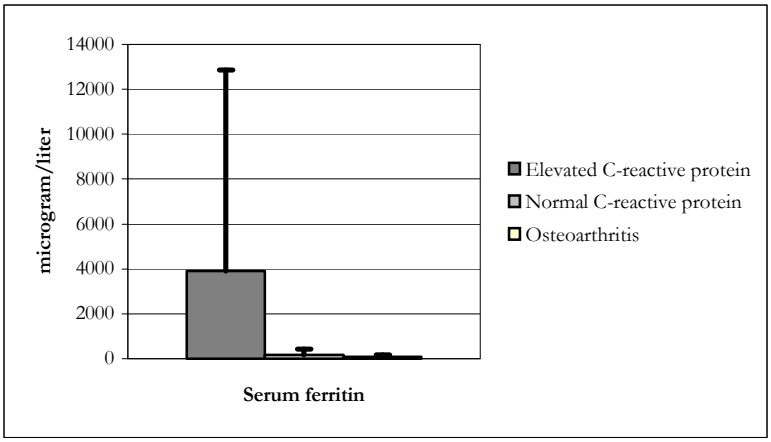

Figure 10a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

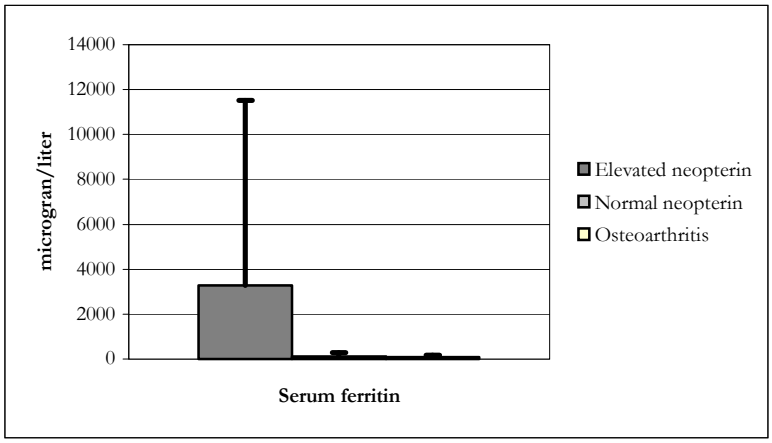

Figure 10b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

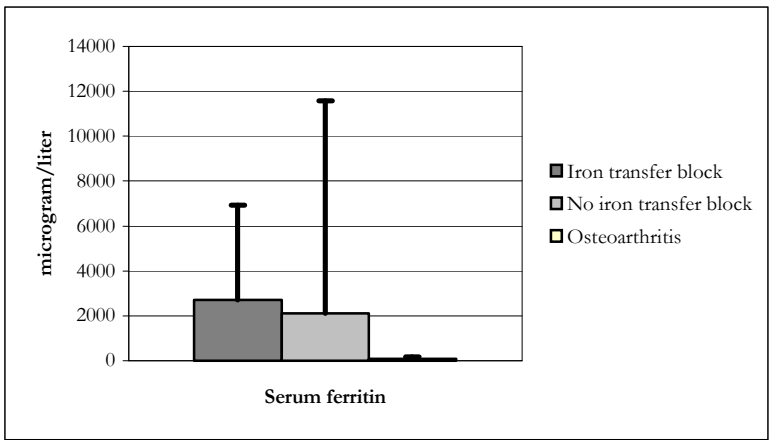

Figure 10c.
Significant higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 10a – 10c. Differences in serum ferritin for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

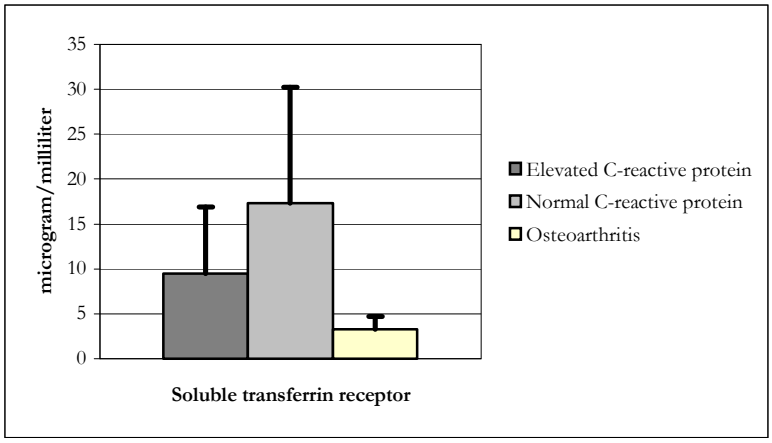

Figure 11a.
Significantly lower for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

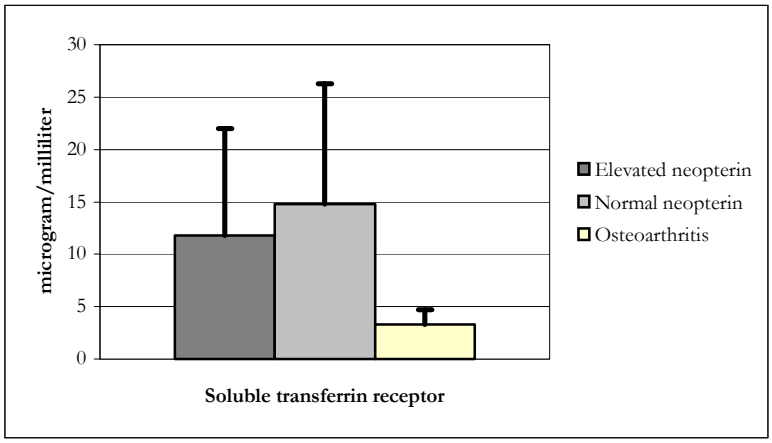

Figure 11b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
Significantly higher for normal neopterin group compared to osteoarthritis group.

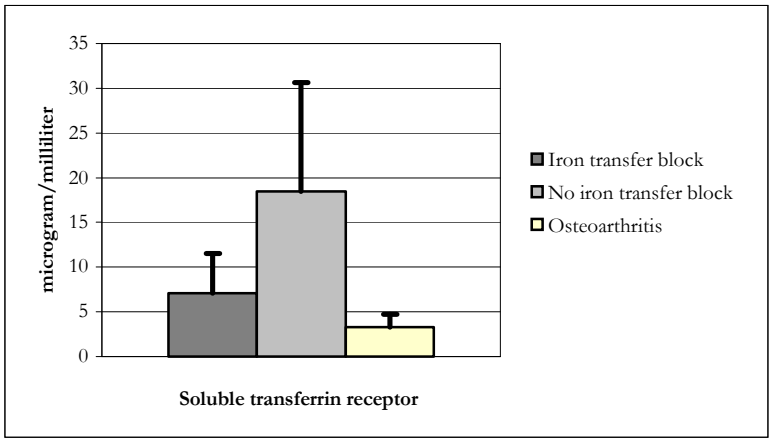

Figure 11c.
Significantly lower for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 11a – 11c. Differences in soluble transferrin receptor for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

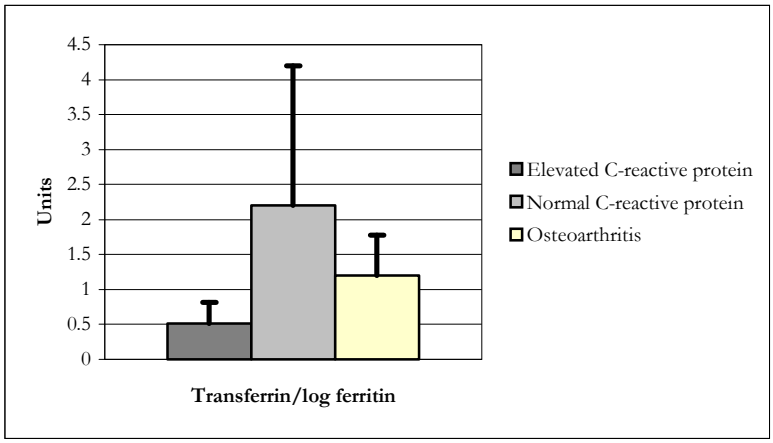

Figure 12a.
Significantly lower for elevated CRP group compared to normal CRP group.
Significantly lower for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

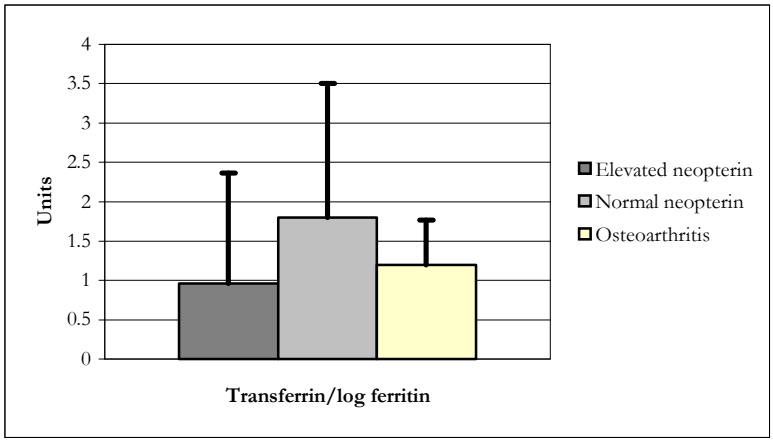

Figure 12b.
Significantly lower for elevated neopterin group compared to normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

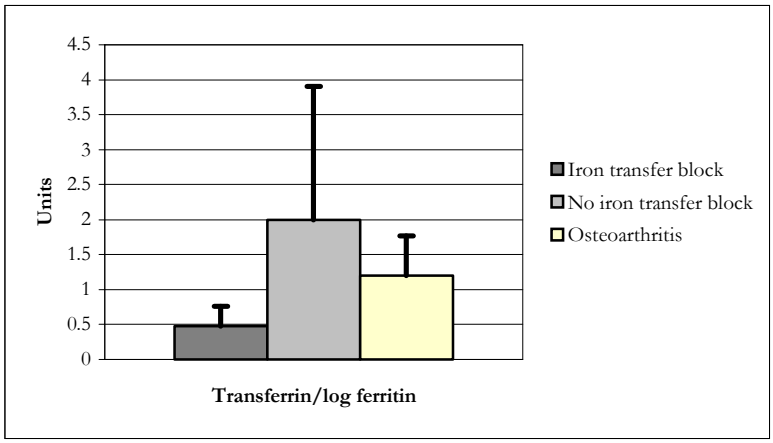

Figure 12c.
Significantly lower for iron transfer block group compared to no iron transfer block group.
Significantly lower for iron transfer block group compared to osteoarthritis group.
Marginally higher for no iron transfer block group compared to osteoarthritis group.

Figure 12a – 12c. Differences in transferrin/log ferritin ratio for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

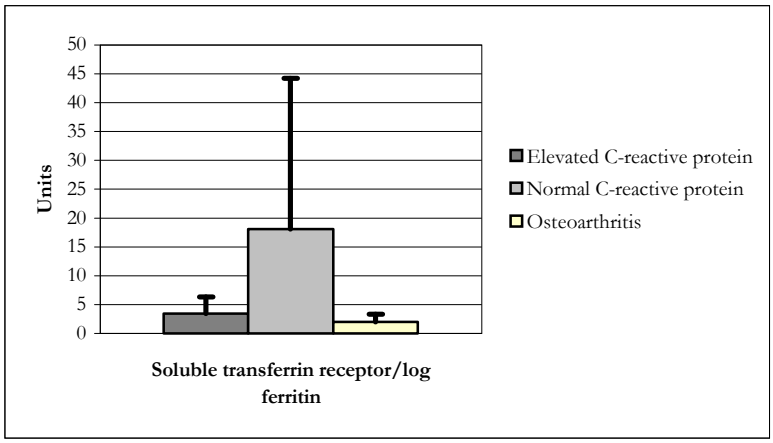

Figure 13a.
Significantly lower for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

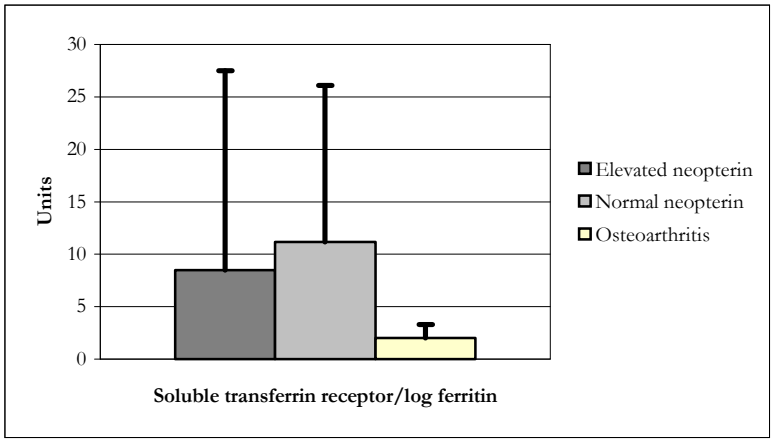

Figure 13b.
Marginally lower for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
Significantly higher for normal neopterin group compared to osteoarthritis group.

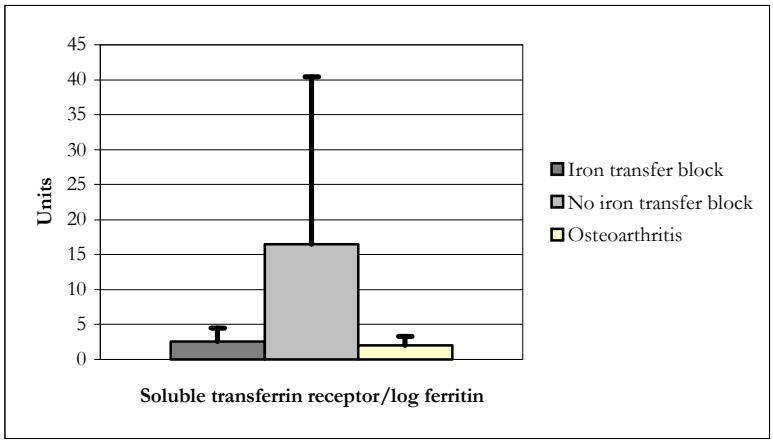

Figure 13c.
Significantly lower for iron transfer block group compared to no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 13a – 13c. Differences in soluble transferrin receptor/log ferritin ratio for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

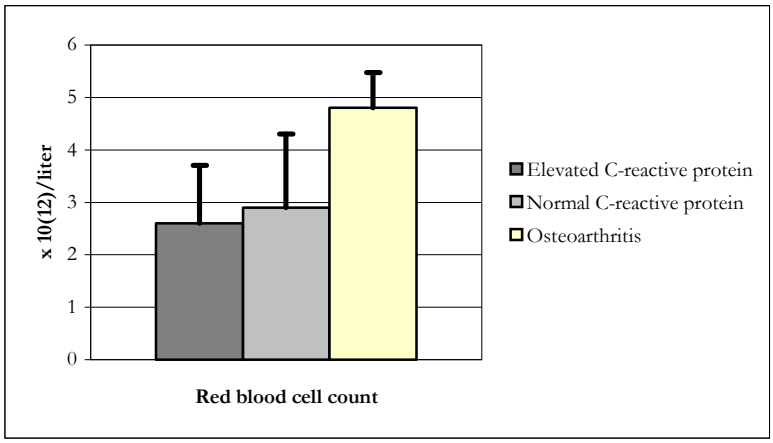

Figure 14a.
Significantly lower for elevated CRP group compared to normal CRP group.
Significantly lower for elevated CRP group compared to osteoarthritis group.
Significantly lower for normal CRP group compared to osteoarthritis group.

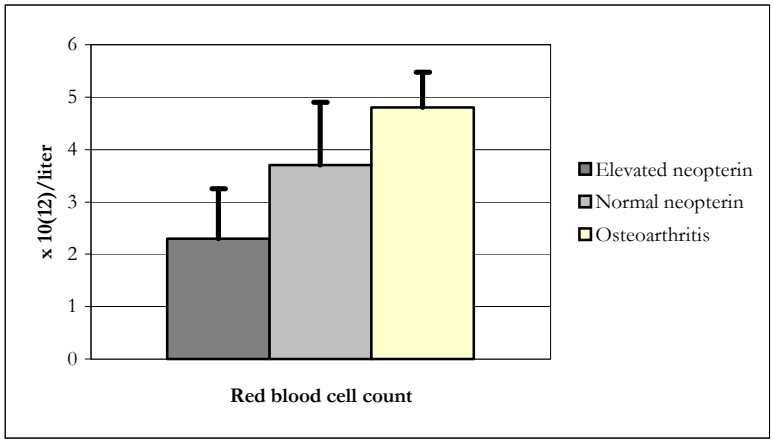

Figure 14b.
Significantly lower for elevated neopterin group compared to normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
Marginally lower for normal neopterin group compared to osteoarthritis group.

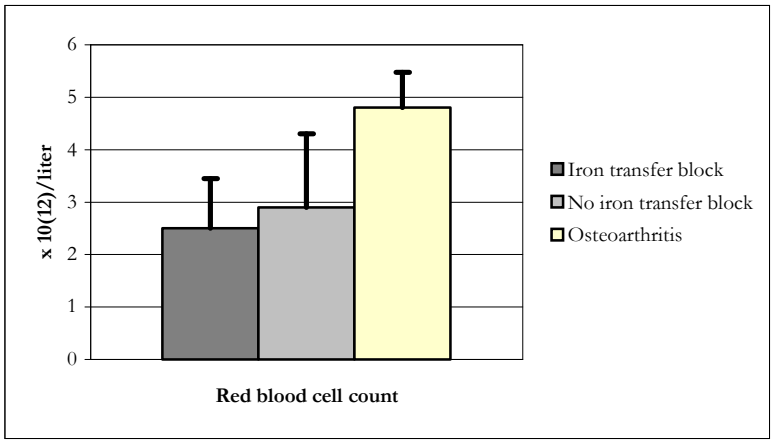

Figure 14c.
No significant difference between iron transfer block group and no iron transfer block group.
Significantly lower for iron transfer block group compared to osteoarthritis group.
Significantly lower for no iron transfer block group compared to osteoarthritis group.

Figure 14a – 14c. Differences in red blood cell count for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

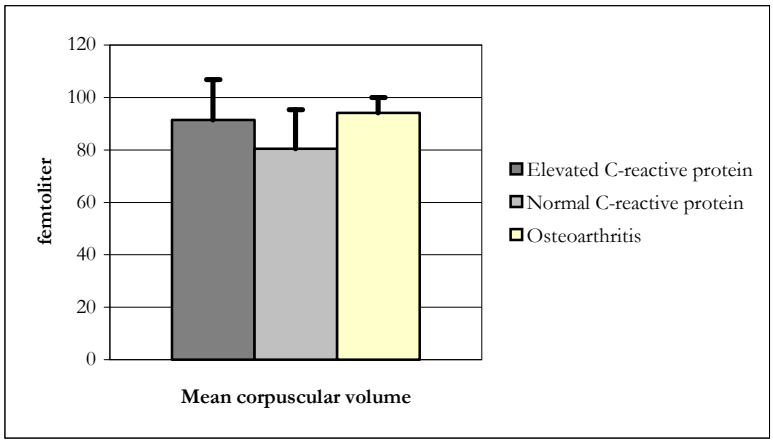

Figure 15a.
Significantly higher for elevated CRP group compared to normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Significantly lower for normal CRP group compared to osteoarthritis group.

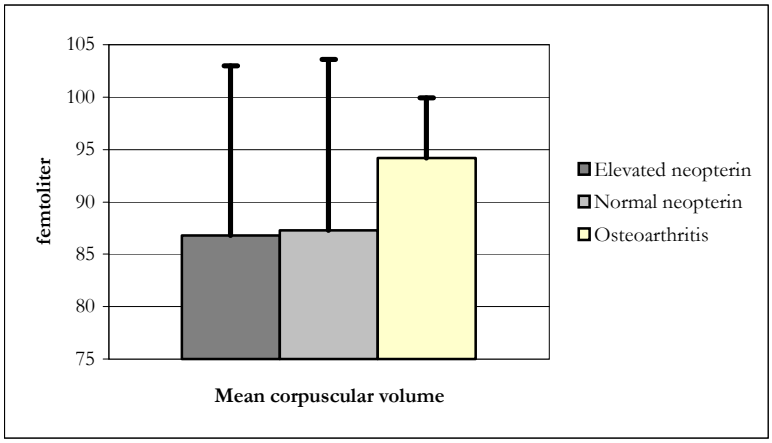

Figure 15b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
Marginally lower for normal neopterin group compared to osteoarthritis group.

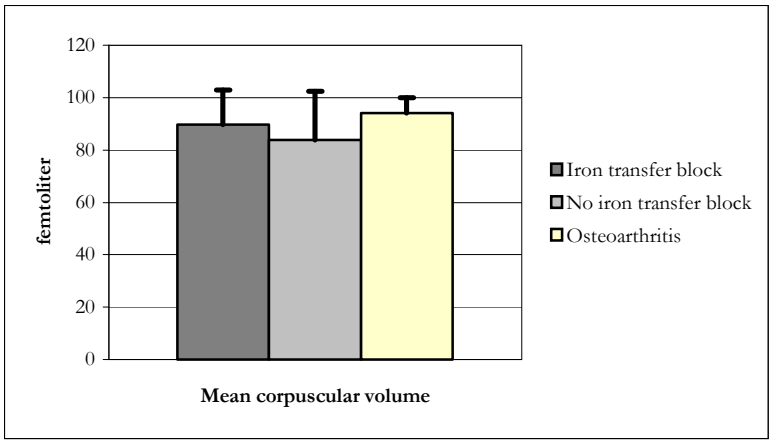

Figure 15c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Significantly lower for no iron transfer block group compared to osteoarthritis group.

Figure 15a – 15c. Differences in mean corpuscular volume for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

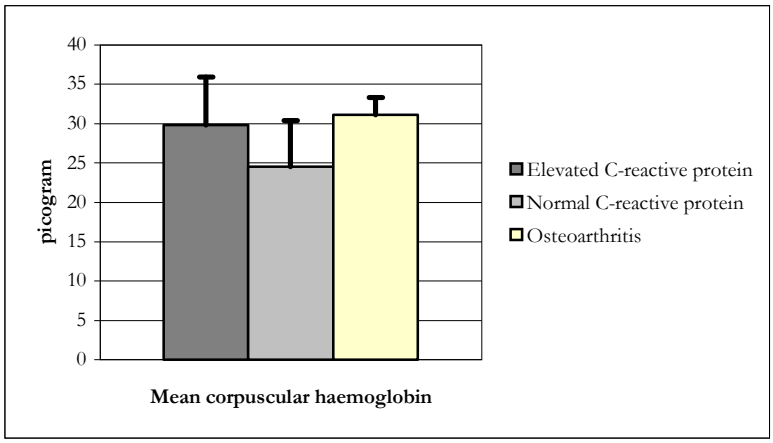

Figure 16a.
Significantly higher for elevated CRP group compared to normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Significantly lower for normal CRP group compared to osteoarthritis group.

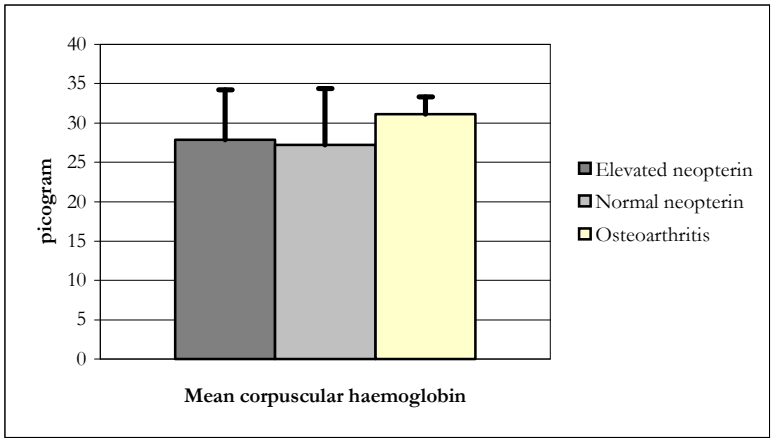

Figure 16b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
Marginally lower for normal neopterin group compared to osteoarthritis group.

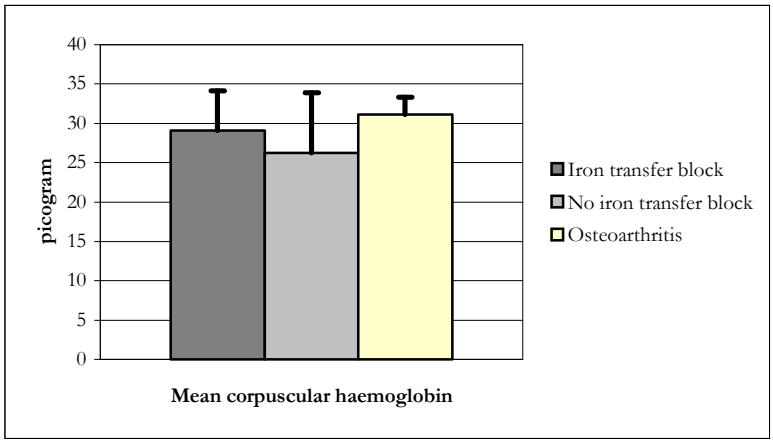

Figure 16c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Significantly lower for no iron transfer block group compared to osteoarthritis group.

Figure 16a – 16c. Differences in mean corpuscular haemoglobin for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

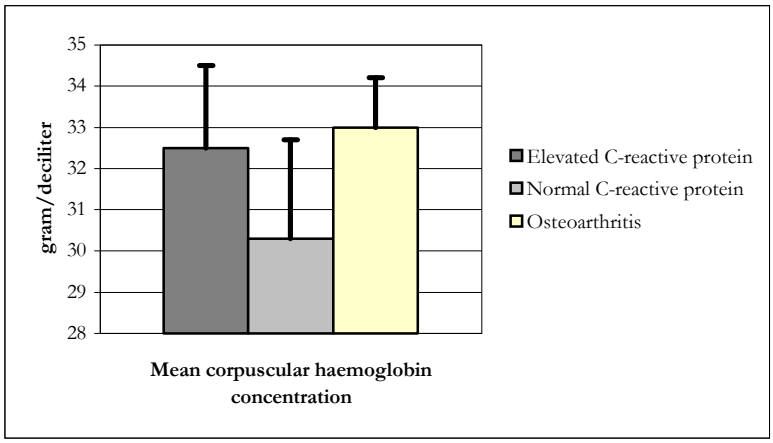

Figure 17a.
Significantly higher for elevated CRP group compared to normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
Significantly lower for normal CRP group compared to osteoarthritis group.

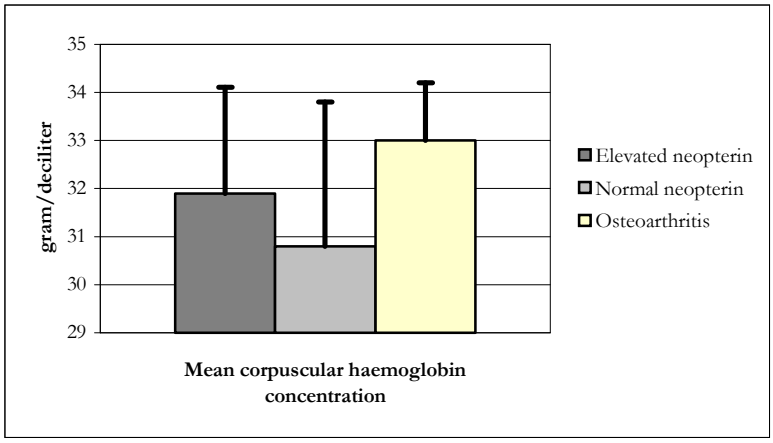

Figure 17b.
No significant difference between elevated neopterin group and normal neopterin group.
Marginally lower for elevated neopterin group compared to osteoarthritis group.
Significantly lower for normal neopterin group compared to osteoarthritis group.

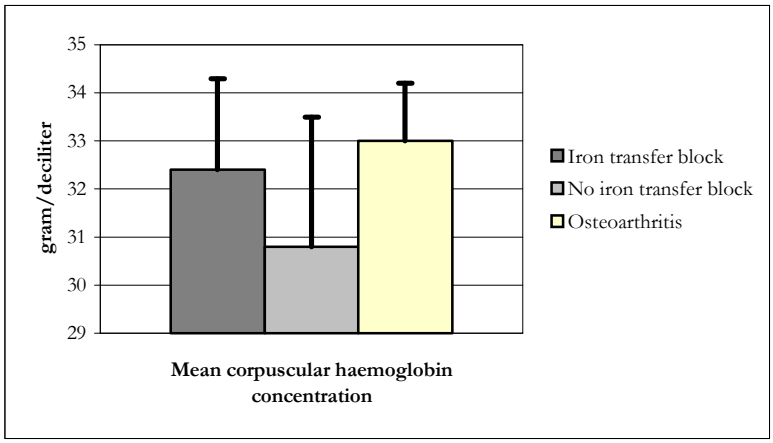

Figure 17c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Significantly lower for no iron transfer block group compared to osteoarthritis group.

Figure 17a – 17c. Differences in mean corpuscular haemoglobin concentration for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

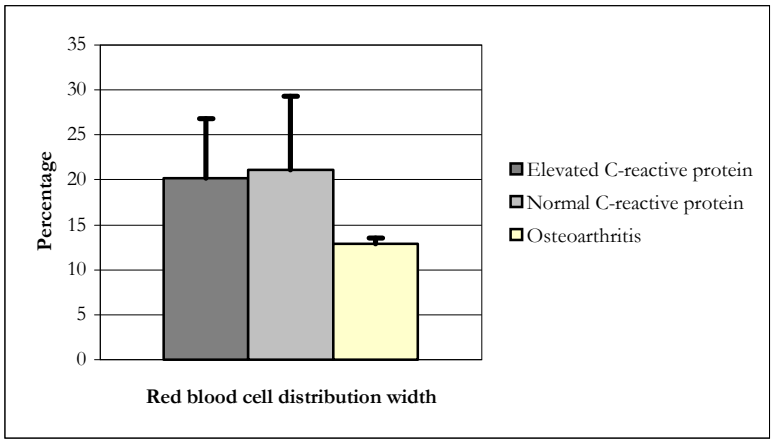

Figure 18a.
No significant difference between elevated CRP group and normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

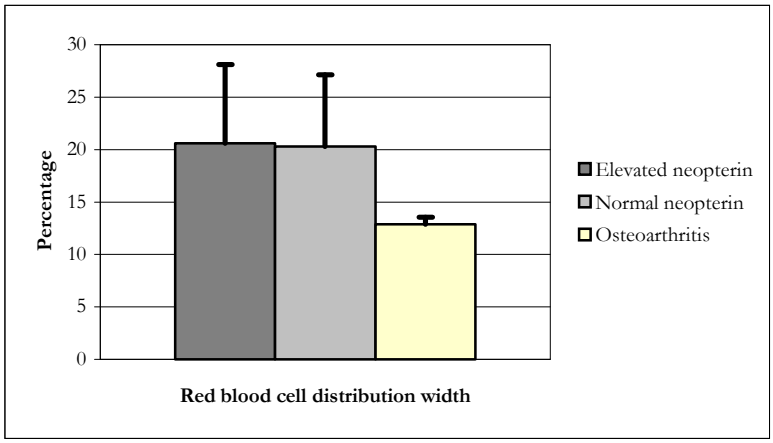

Figure 18b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
Significantly higher for normal neopterin group compared to osteoarthritis group.

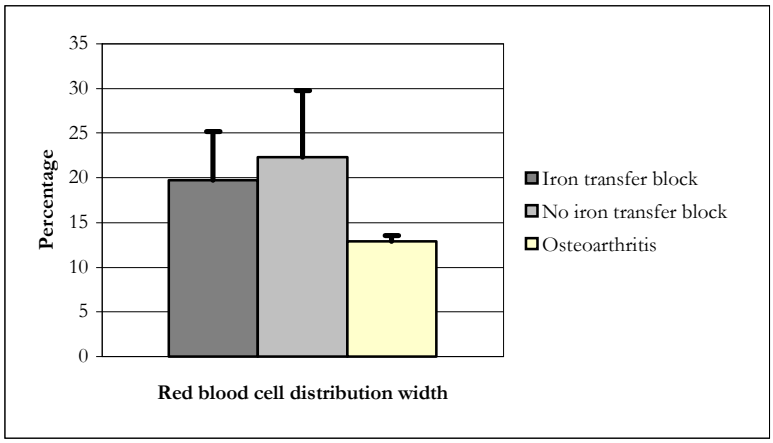

Figure 18c.
No significant difference between iron transfer block group and no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 18a – 18c. Differences in red blood cell distribution width for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

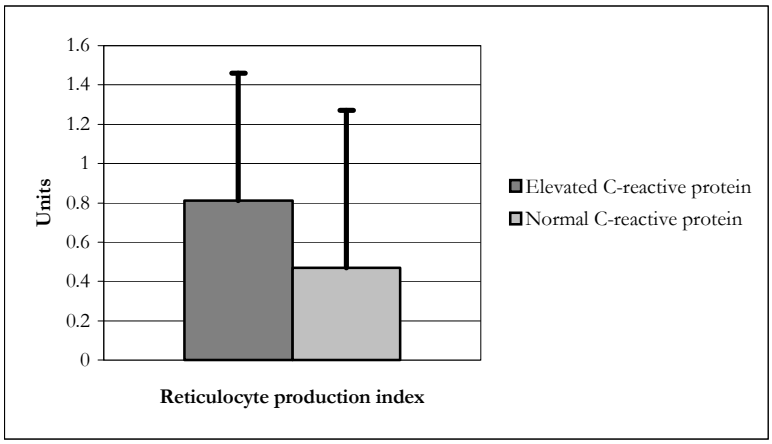

Figure 19a.
Significantly higher for elevated CRP group compared to normal CRP group.

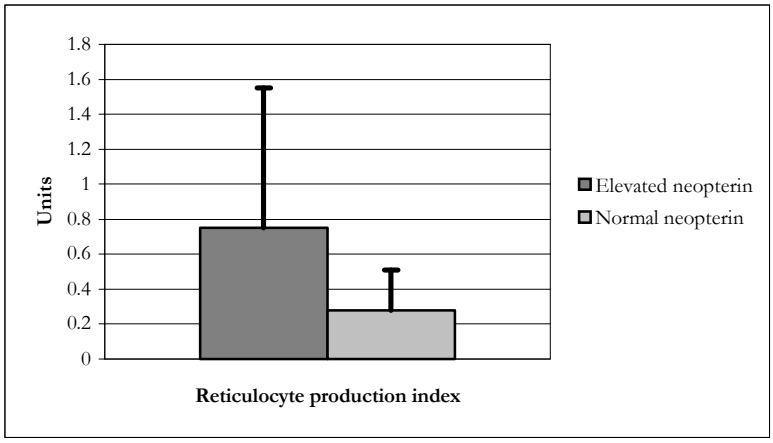

Figure 19b.
Significantly higher for elevated neopterin group compared to normal neopterin group.

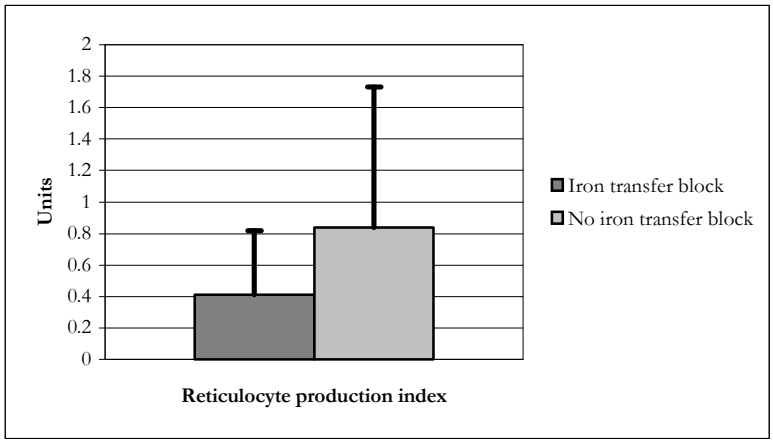

Figure 19c.
No significant difference between iron transfer block group and no iron transfer block group.

Figure 19a – 19c. Differences in reticulocyte production index for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision. The reticulocyte production index was not determined for the osteoarthritis patients.

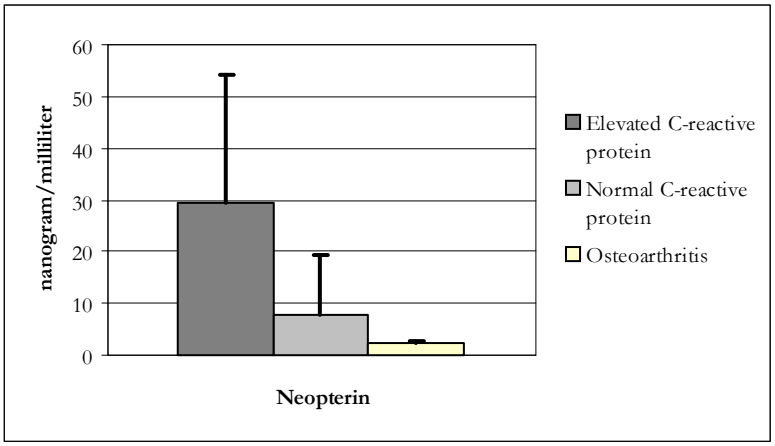

Figure 20a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

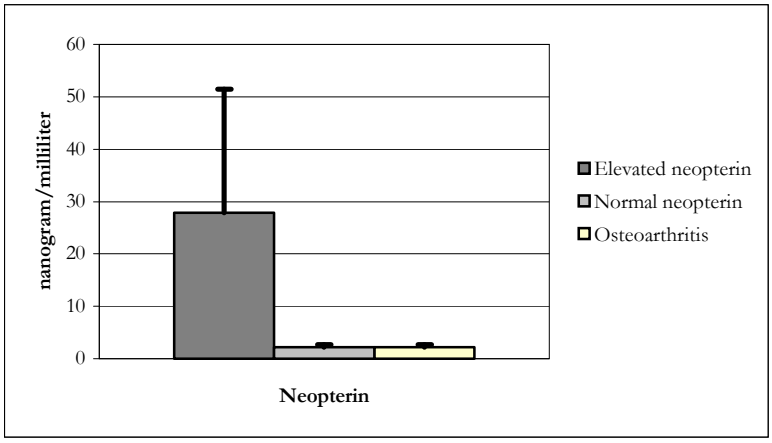

Figure 20b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

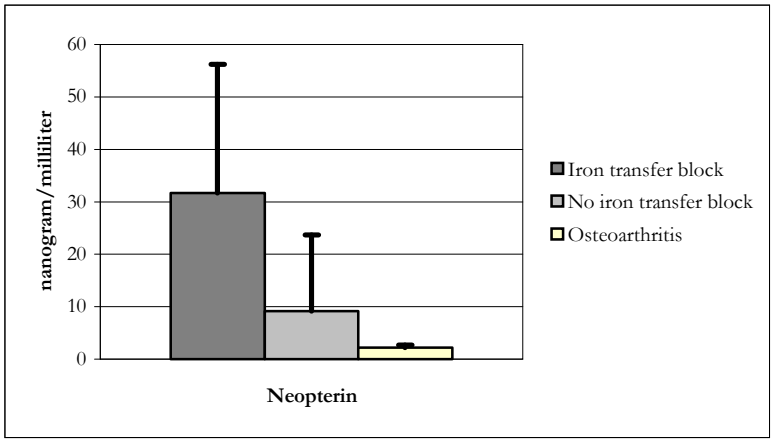

Figure 20c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 20a – 20c. Differences in neopterin for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

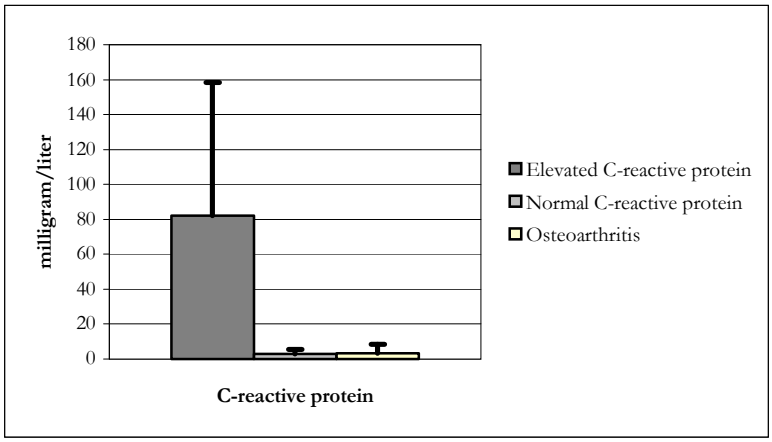

Figure 21a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

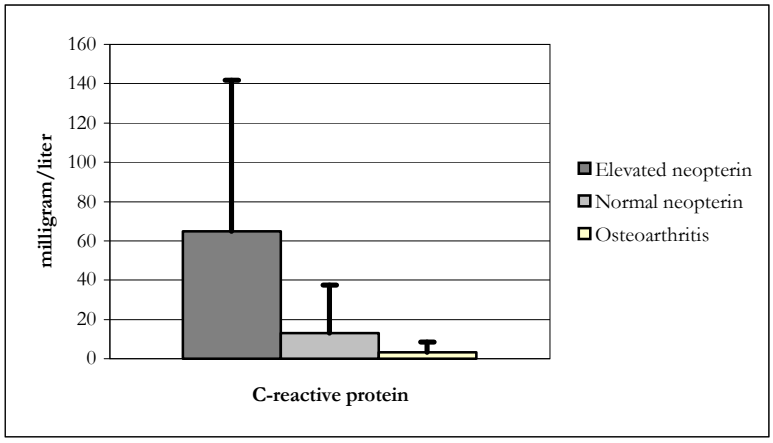

Figure 21b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

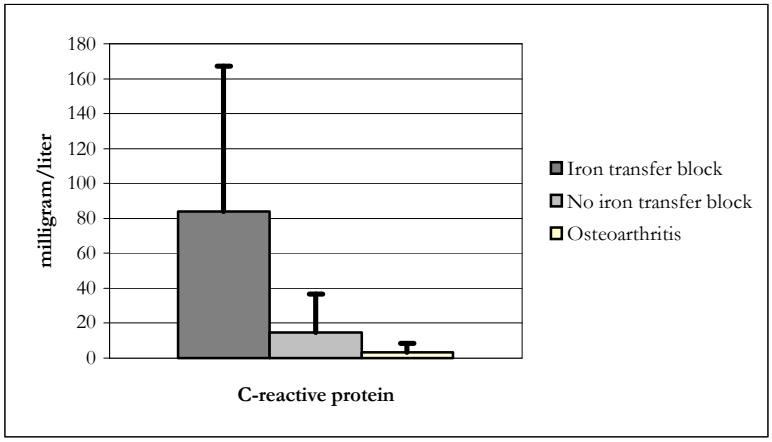

Figure 21c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 21a – 21c. Differences in C-reactive protein for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

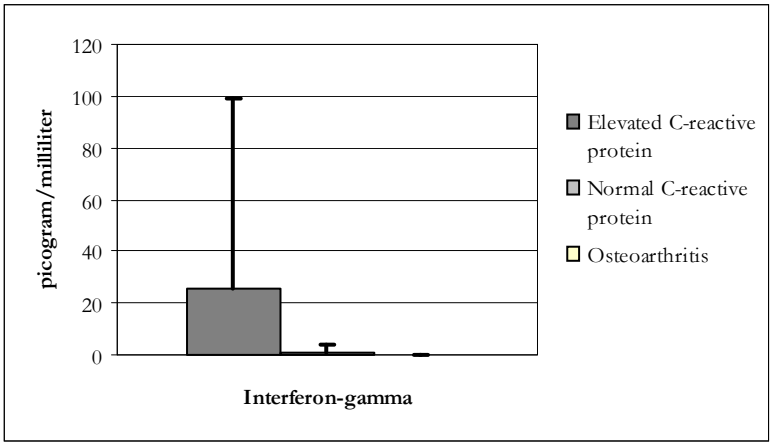

Figure 22a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

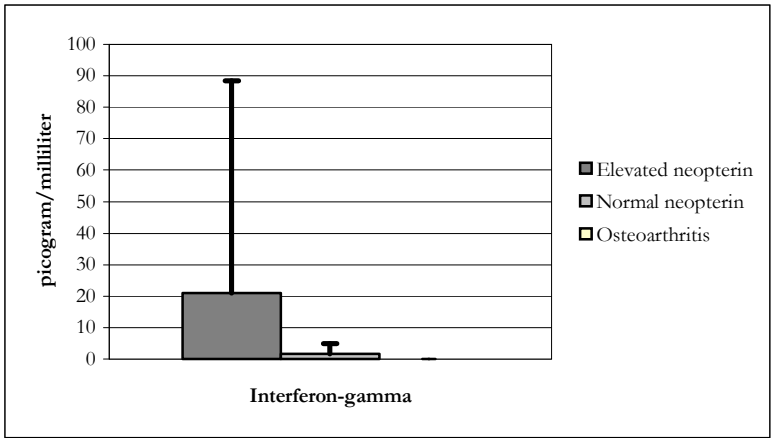

Figure 22b.
Marginally higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

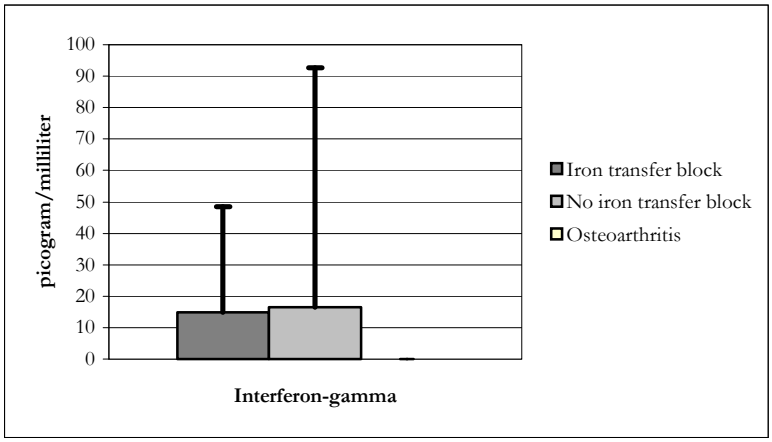

Figure 22c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 22a – 22c. Differences in interferon-γ for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

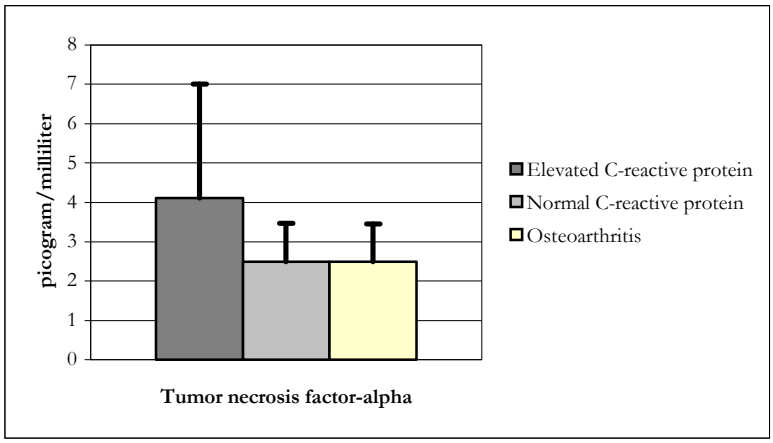

Figure 23a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

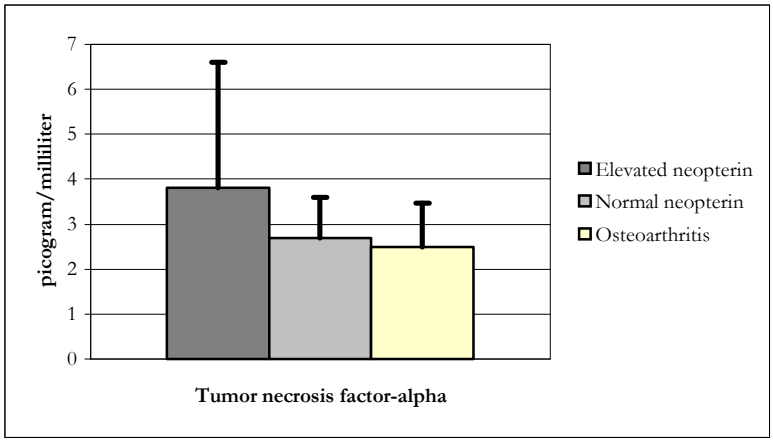

Figure 23b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

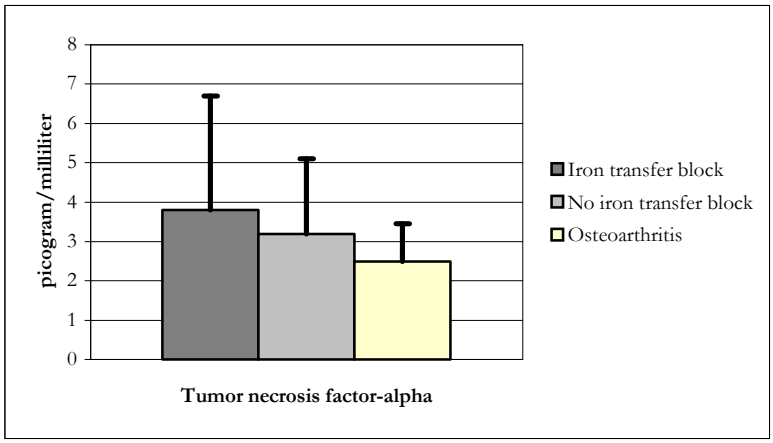

Figure 23c.
No significant difference between iron transfer block group and no iron transfer block group.
Marginally higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 23a – 23c. Differences in tumor necrosis factor-α for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

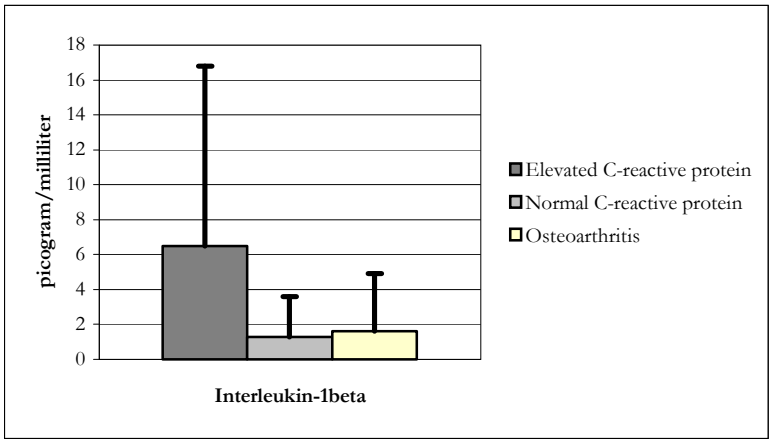

Figure 24a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

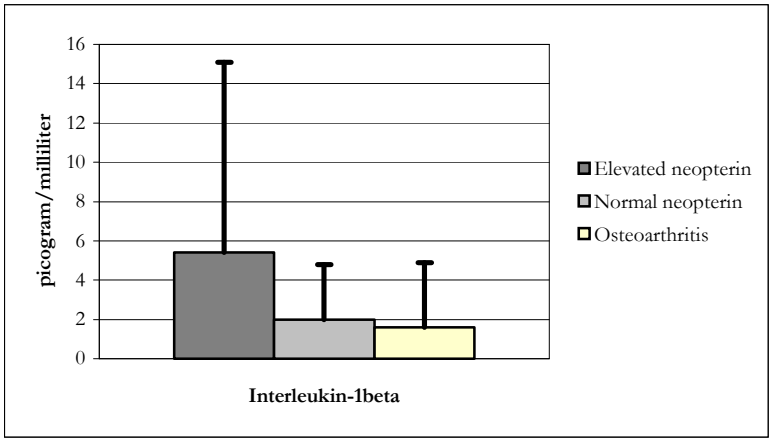

Figure 24b.
Marginally higher for elevated neopterin group compared to normal neopterin group.
Marginally higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

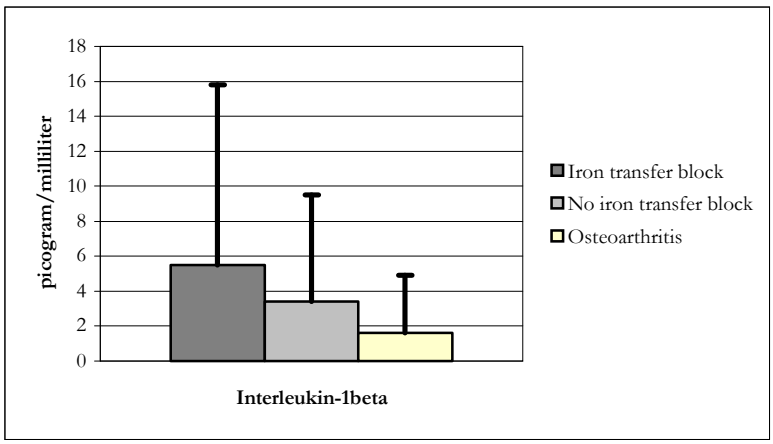

Figure 24c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 24a – 24c. Differences in interleukin-1β for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

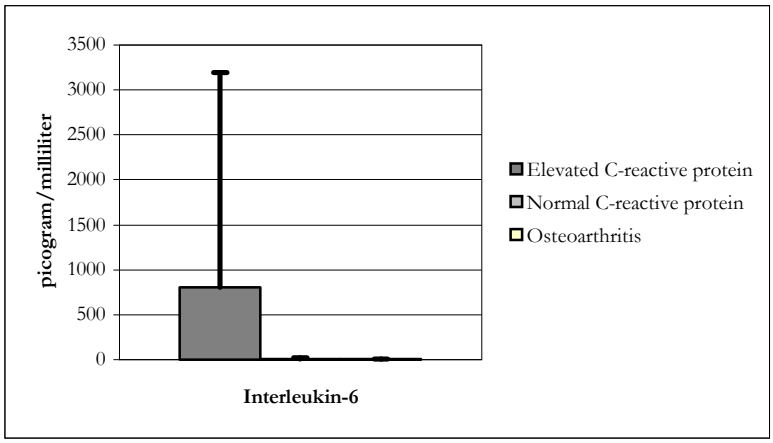

Figure 25a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

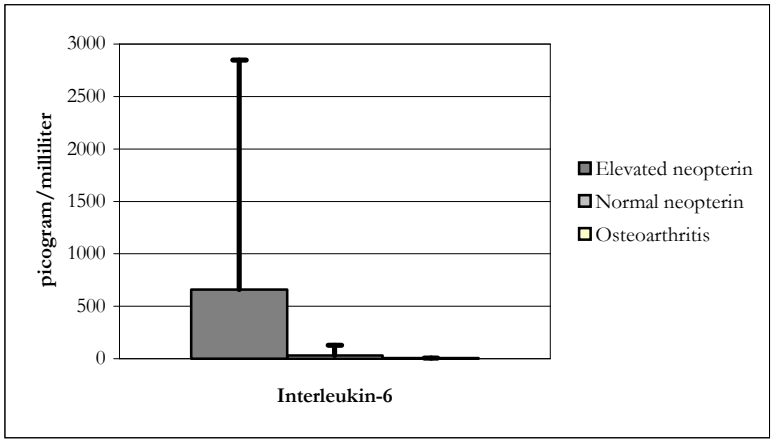

Figure 25b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

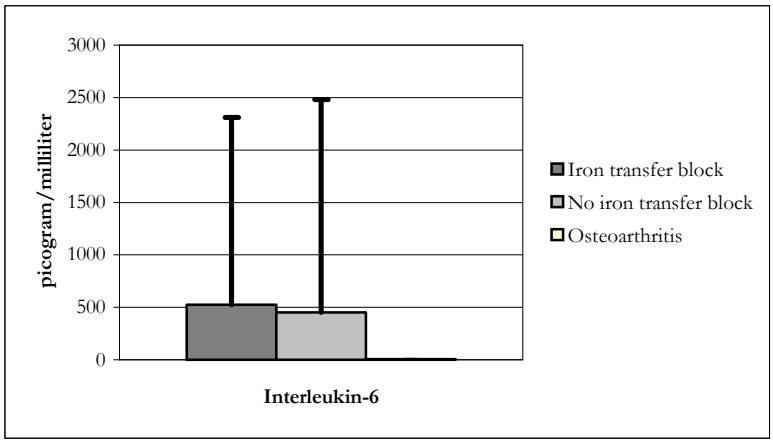

Figure 25c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 25a – 25c. Differences in interleukin-6 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

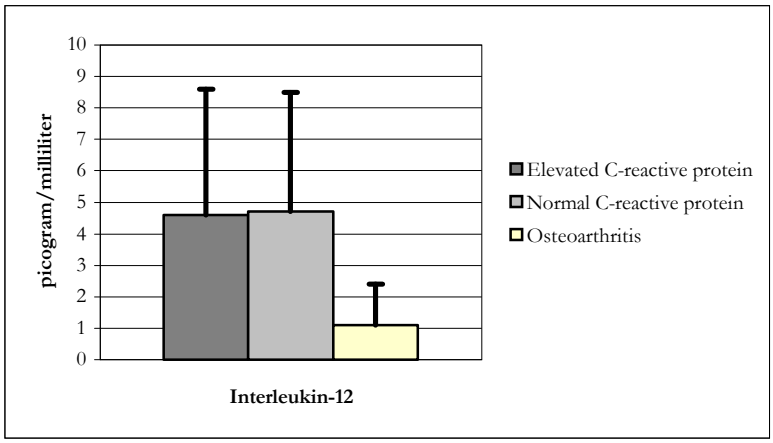

Figure 26a.
No significant difference between elevated CRP group and normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

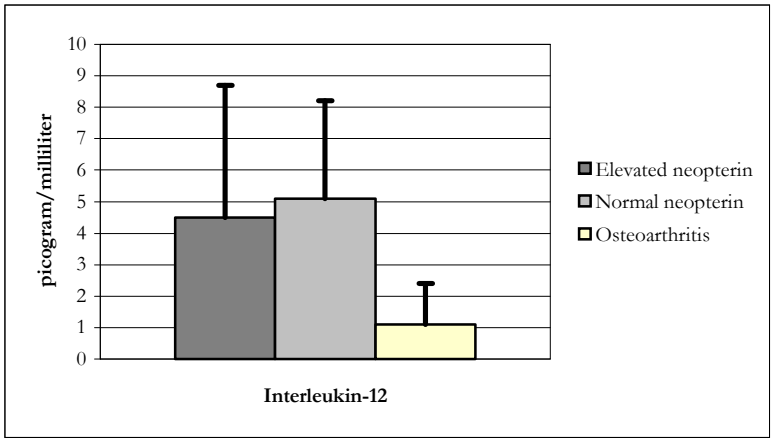

Figure 26b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.

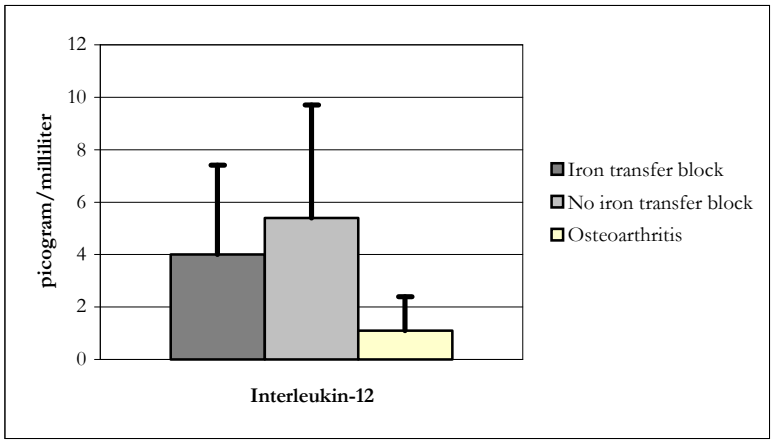

Figure 26c.
No significant difference between iron transfer block group and no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 26a – 26c. Differences in interleukin-12 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

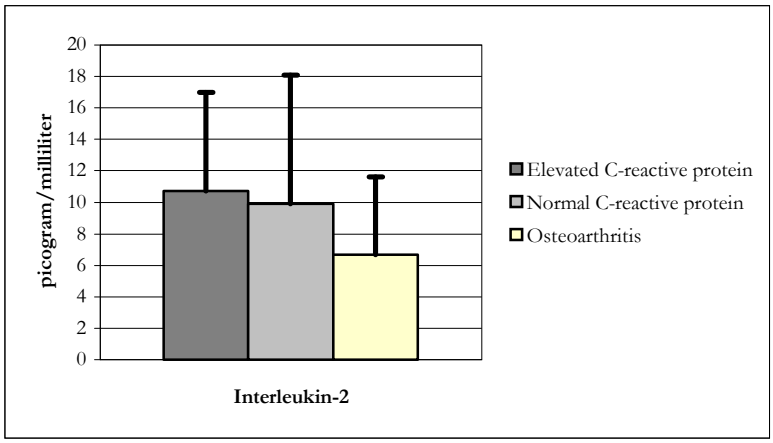

Figure 27a.
No significant difference between elevated CRP group and normal CRP group.
Marginally higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

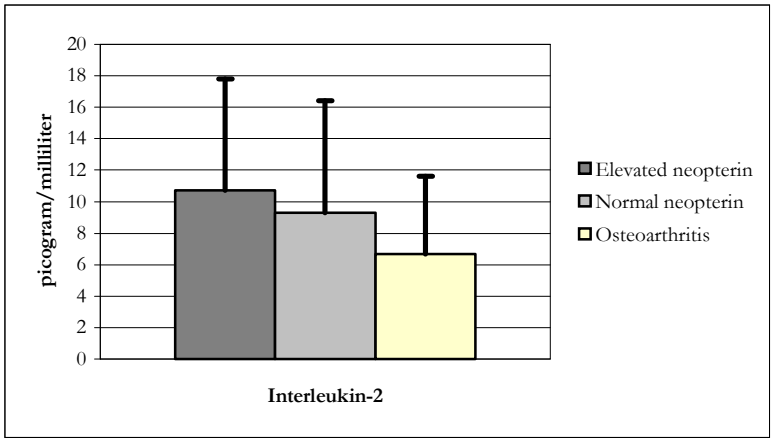

Figure 27b.
No significant difference between elevated neopterin group and normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

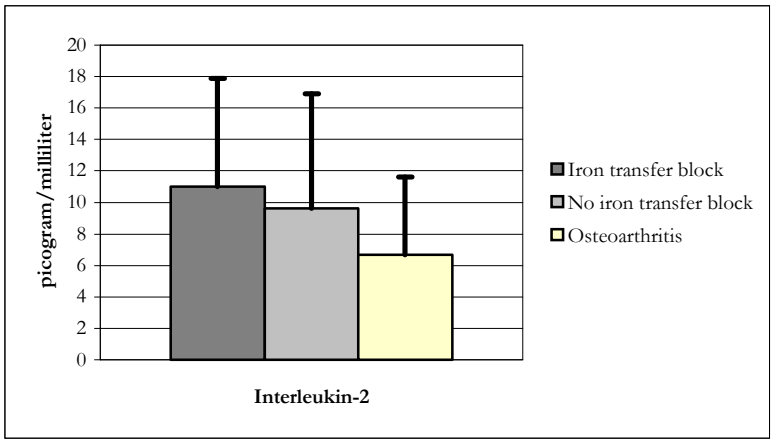

Figure 27c.
No significant difference between iron transfer block group and no iron transfer block group**.**
Significantly higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 27a – 27c. Differences in interleukin-2 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

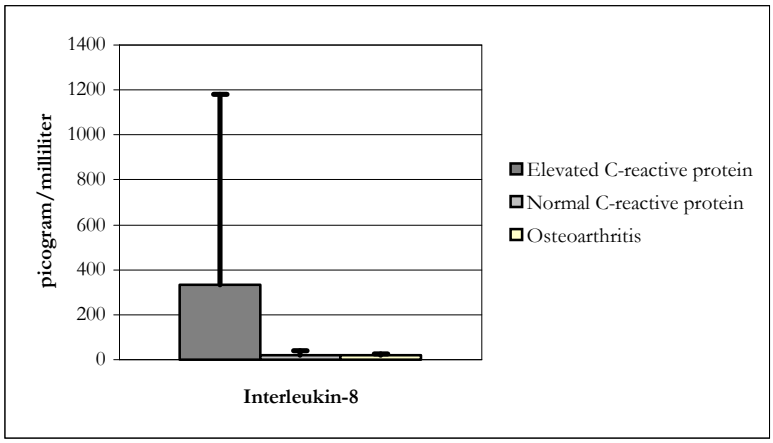

Figure 28a.
Significantly higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

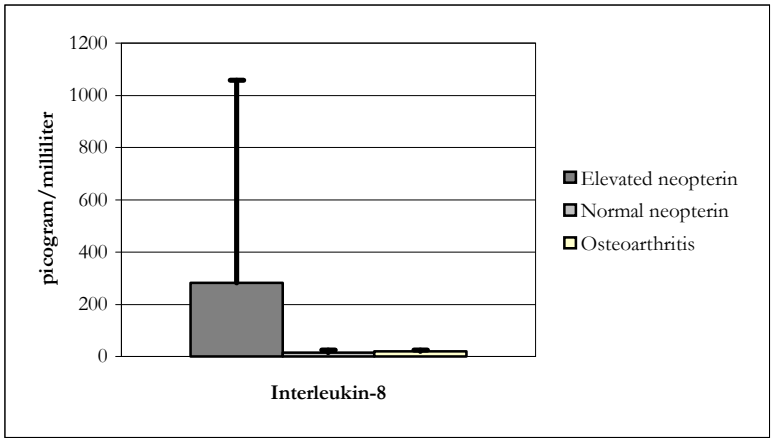

Figure 28b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

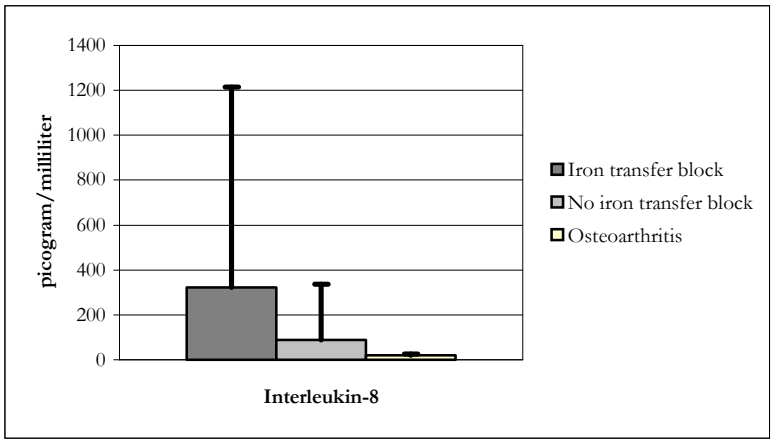

Figure 28c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 28a – 28c. Differences in interleukin-8 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

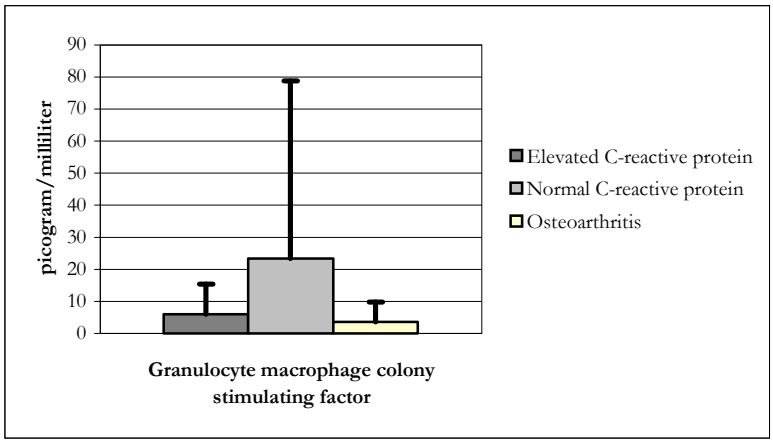

Figure 29a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

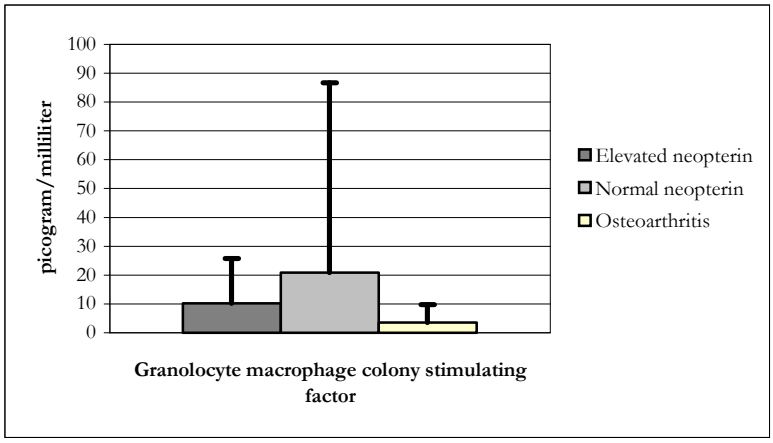

Figure 29b.
No significant difference between elevated neopterin group and normal neopterin group.
Marginally higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

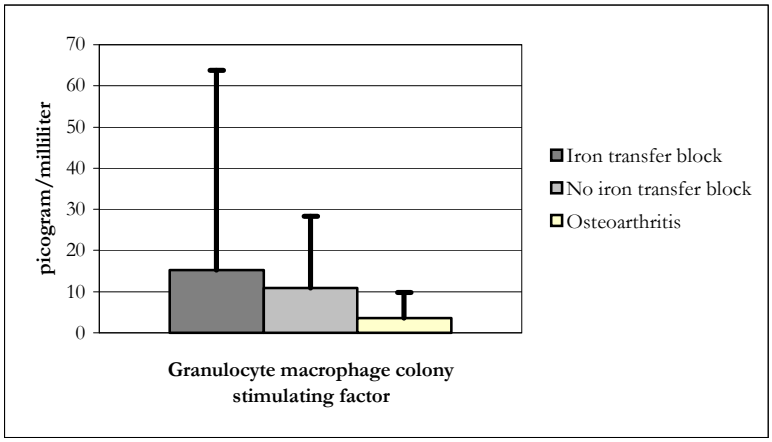

Figure 29c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
Marginally higher for no iron transfer block group compared to osteoarthritis group.

Figure 29a – 29c. Differences in granulocyte macrophage colony stimulating factor for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

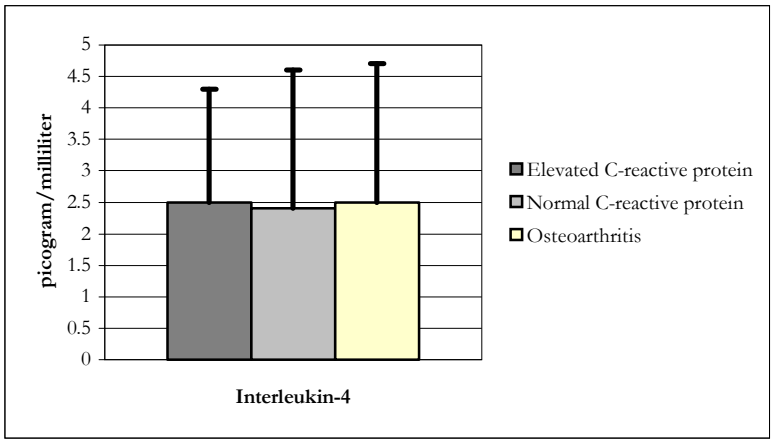

Figure 30a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

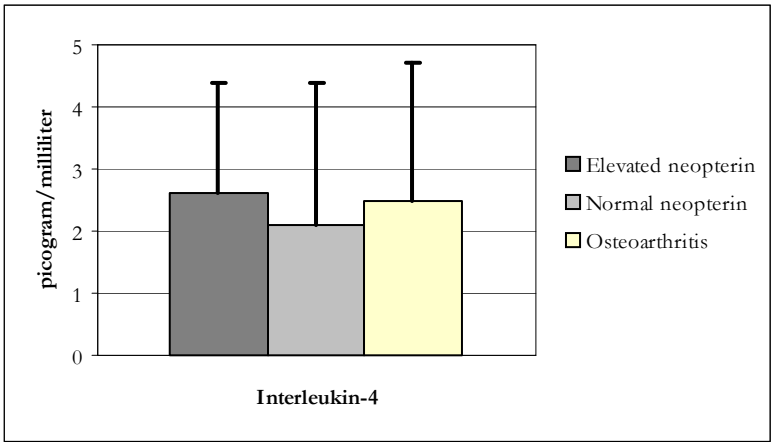

Figure 30b.
No significant difference between elevated neopterin group and normal neopterin group.
No significant difference between elevated neopterin group and osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

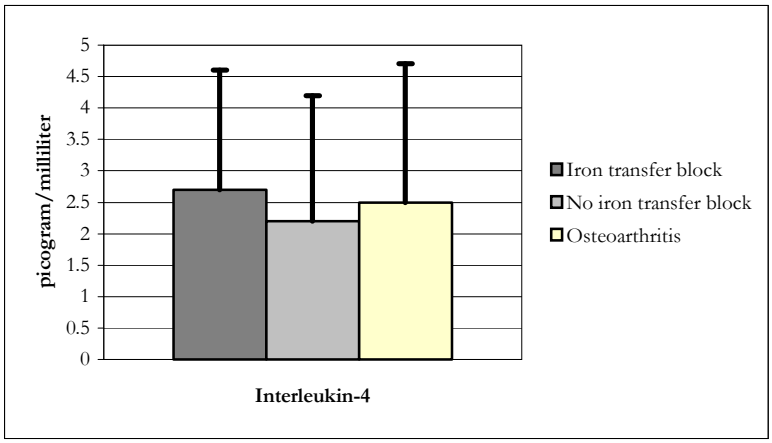

Figure 30c.
No significant difference between iron transfer block group and no iron transfer block group.
No significant difference between iron transfer block group and osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 30a – 30c. Differences in interleukin-4 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

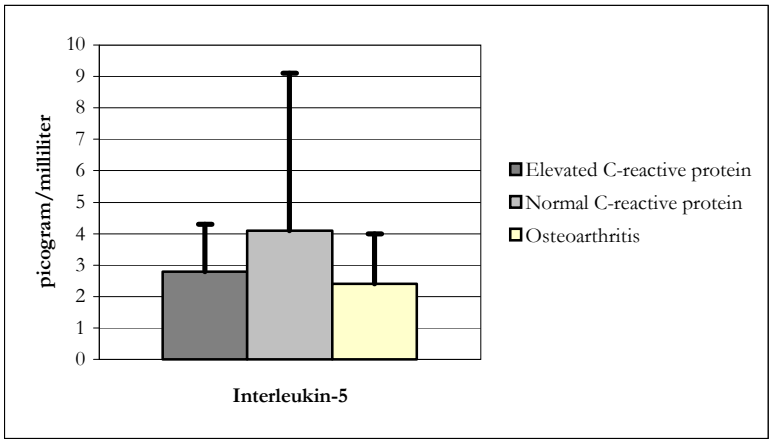

Figure 31a.
No significant difference between elevated CRP group and normal CRP group.
No significant difference between elevated CRP group and osteoarthritis group.
No significant difference between normal CRP group and osteoarthritis group.

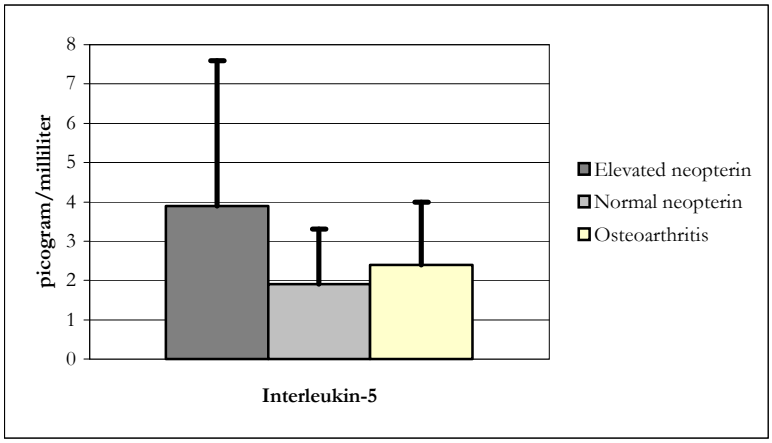

Figure 31b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Marginally higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

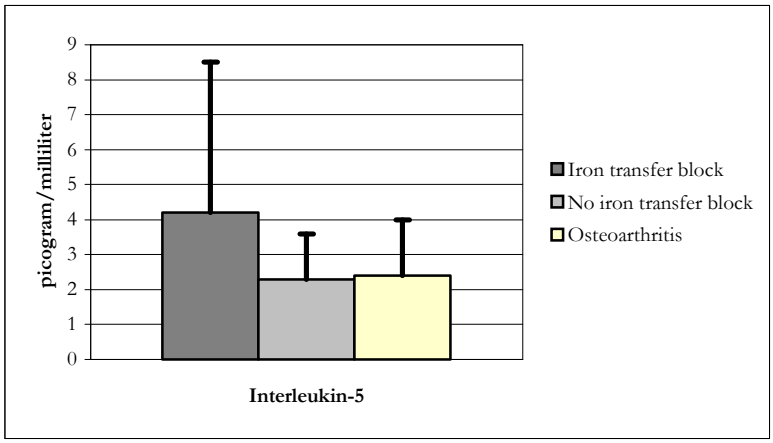

Figure 31c.
Significantly higher for iron transfer block group compared to no iron transfer block group.
Marginally higher for iron transfer block group compared to osteoarthritis group.
No significant difference between no iron transfer block group and osteoarthritis group.

Figure 31a – 31c. Differences in interleukin-5 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

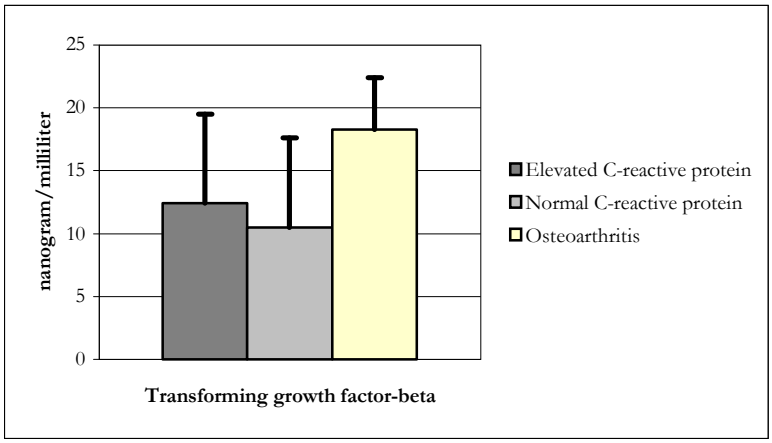

Figure 32a.
No significant difference between elevated CRP group and normal CRP group.
Significantly lower for elevated CRP group compared to osteoarthritis group.
Significantly lower for normal CRP group compared to osteoarthritis group.

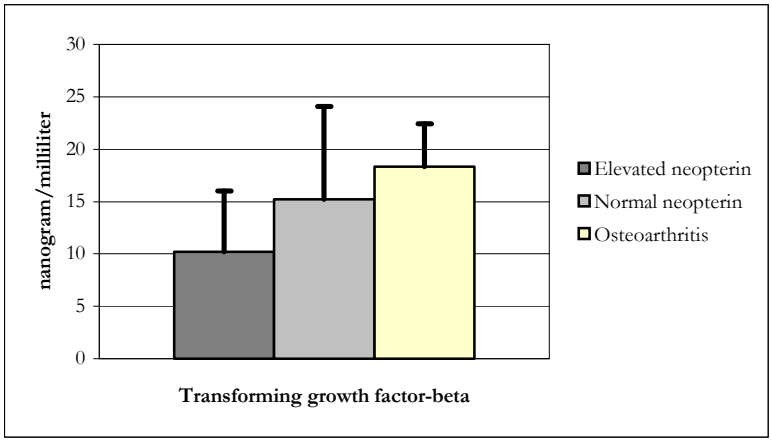

Figure 32b.
Marginally lower for elevated neopterin group compared to normal neopterin group.
Significantly lower for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

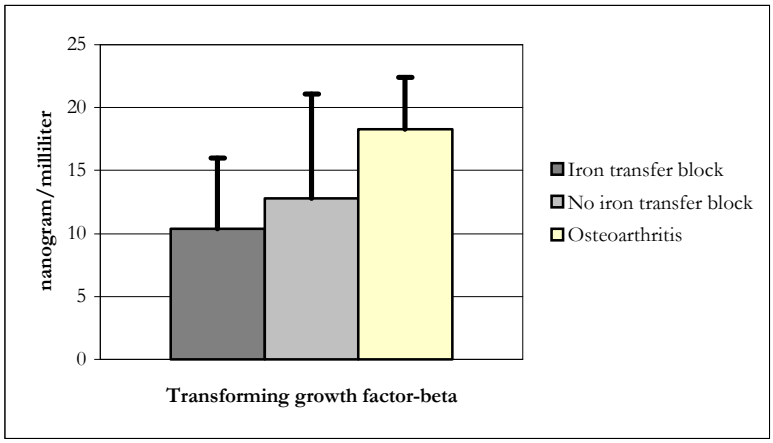

Figure 32c.
No significant difference between iron transfer block group and no iron transfer block group.
Significantly lower for iron transfer block group compared to osteoarthritis group.
Significantly lower for no iron transfer block group compared to osteoarthritis group.

Figure 32a – 32c. Differences in transforming growth factor-β for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

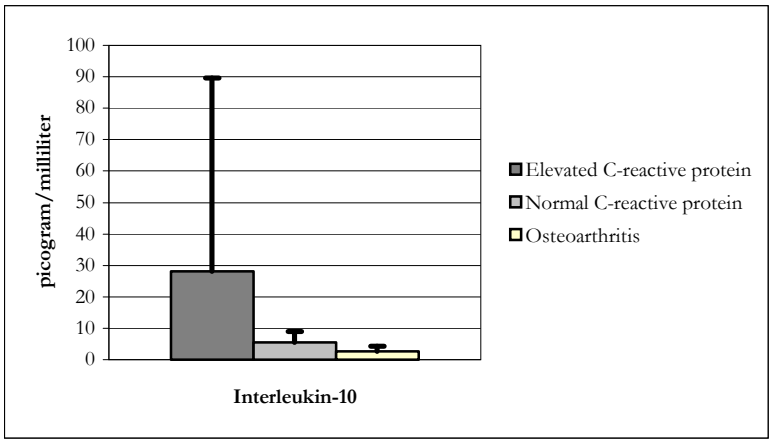

Figure 33a.
Marginally higher for elevated CRP group compared to normal CRP group.
Significantly higher for elevated CRP group compared to osteoarthritis group.
Significantly higher for normal CRP group compared to osteoarthritis group.

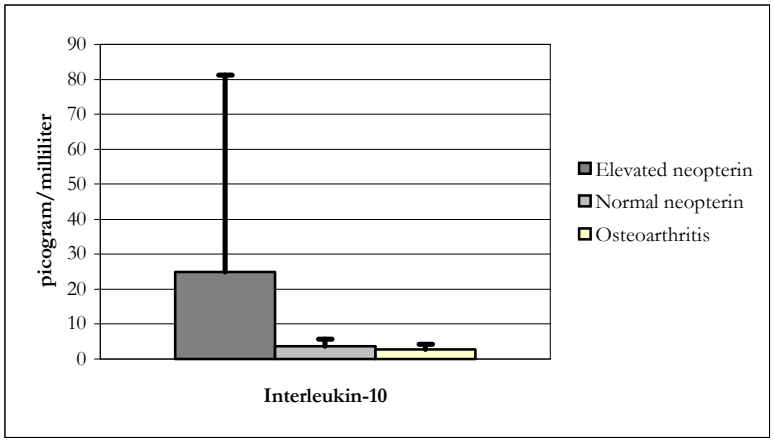

Figure 33b.
Significantly higher for elevated neopterin group compared to normal neopterin group.
Significantly higher for elevated neopterin group compared to osteoarthritis group.
No significant difference between normal neopterin group and osteoarthritis group.

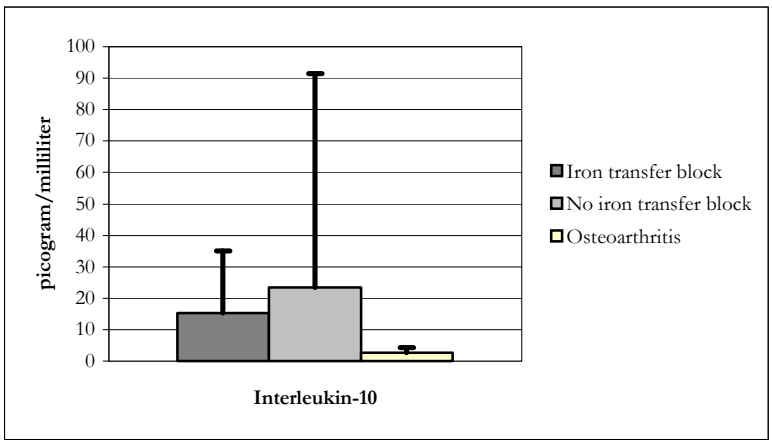

Figure 33c.
No significant difference between iron transfer block group and no iron transfer block group.
Significantly higher for iron transfer block group compared to osteoarthritis group.
Significantly higher for no iron transfer block group compared to osteoarthritis group.

Figure 33a – 33c. Differences in interleukin-10 for the C-reactive protein subdivision, the neopterin subdivision and the iron transfer block subdivision.

5) Correlations in the different subgroups of the Kalafong patients and the group of osteoarthritis patients

5.1) Correlations in the group of Kalafong patients with normal C-reactive protein

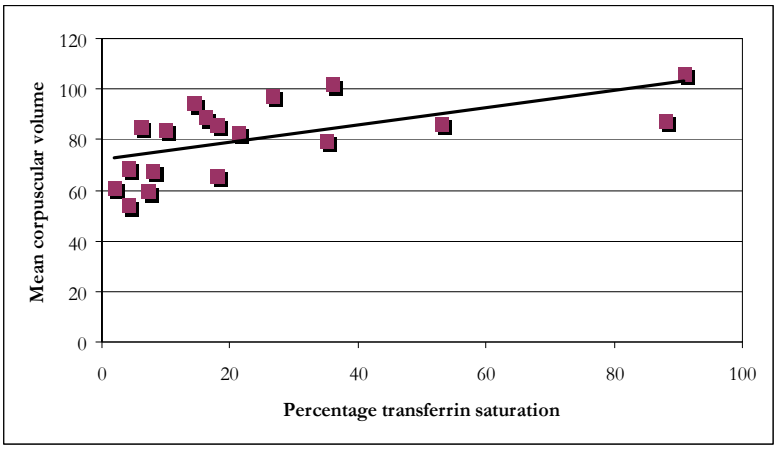

Figure 1.
Correlation between the percentage transferrin saturation and the mean corpuscular volume in the group of Kalafong patients with normal CRP (r-value = 0.62, p-value = 0.005).

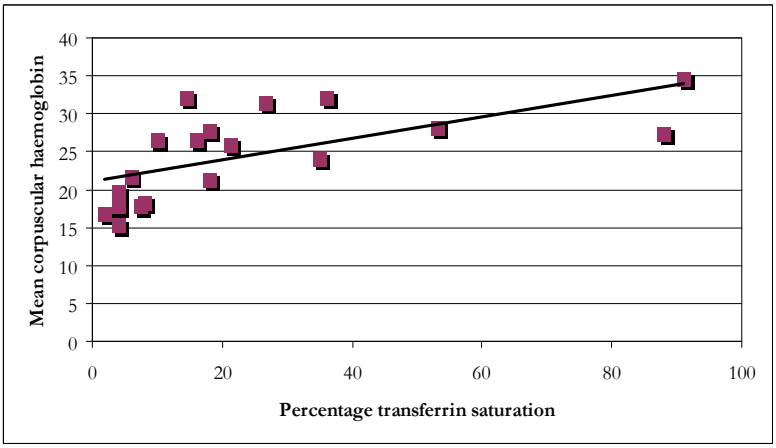

Figure 2.
Correlation between the percentage transferrin saturation and the mean corpuscular haemoglobin in the group of Kalafong patients with normal CRP (r-value = 0.64, p-value = 0.003).

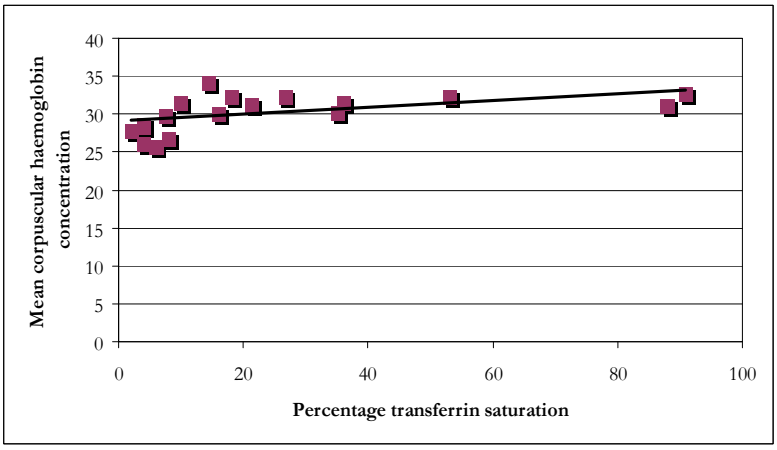

Figure 3.
Correlation between the percentage transferrin saturation and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal CRP (r-value = 0.49, p-value = 0.033).

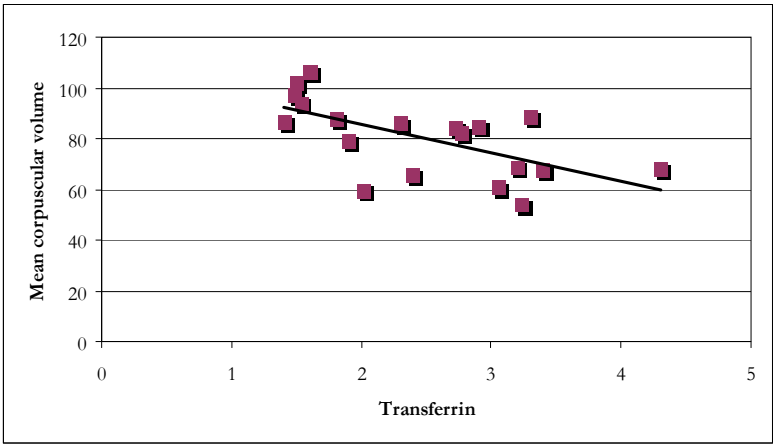

Figure 4.
Correlation between transferrin and the mean corpuscular volume in the group of Kalafong patients with normal CRP (r-value = 0.62, p-value = 0.005).

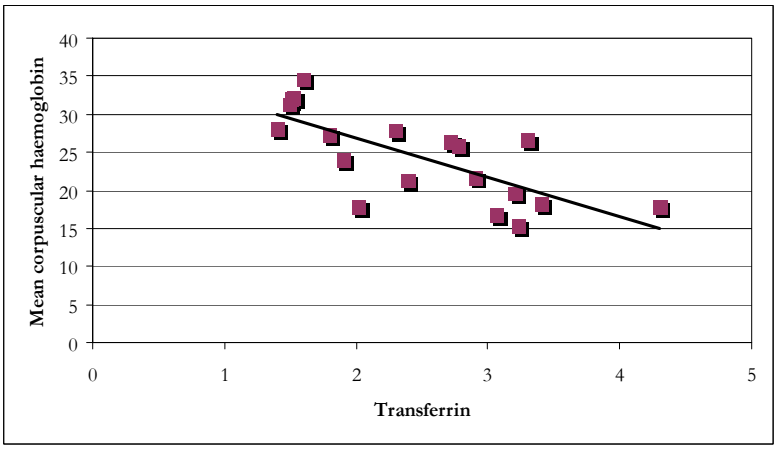

Figure 5.
Correlation between transferrin and the mean corpuscular haemoglobin in the group of Kalafong patients with normal CRP (r-value = 0.74, p-value = 0.0003).

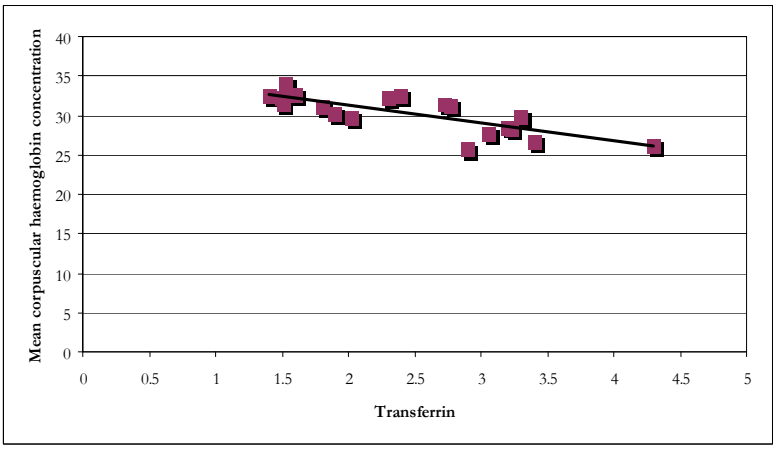

Figure 6.
Correlation between transferrin and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal CRP (r-value = 0.79, p-value = 0.0001).

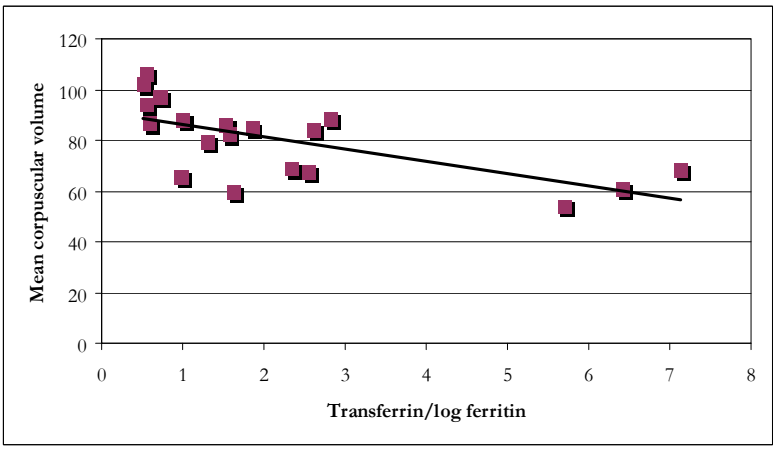

Figure 7.
Correlation between the transferrin/log ferritin ratio and the mean corpuscular volume in the group of Kalafong patients with normal CRP (r-value = 0.66, p-value = 0.002).

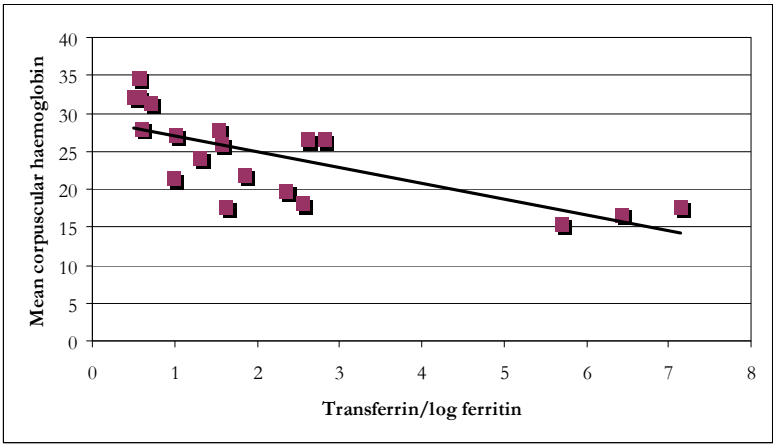

Figure 8.
Correlation between the transferrin/log ferritin ratio and the mean corpuscular haemoglobin in the group of Kalafong patients with normal CRP (r-value = 0.72, p-value = 0.0005).

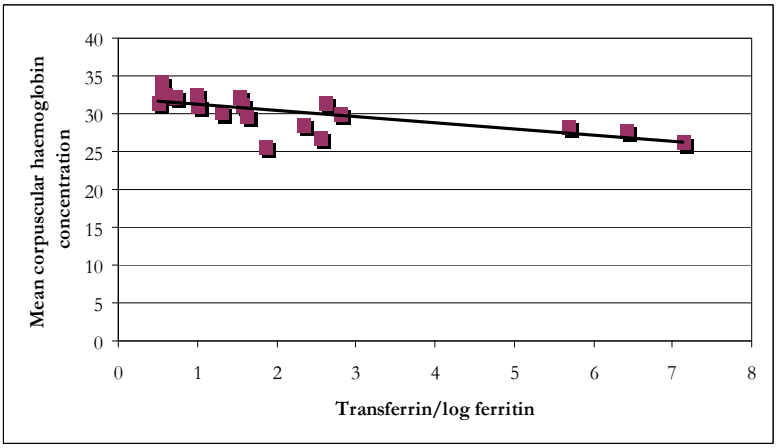

Figure 9.
Correlation between the transferrin/log ferritin ratio and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal CRP (r-value = 0.70, p-value = 0.001).

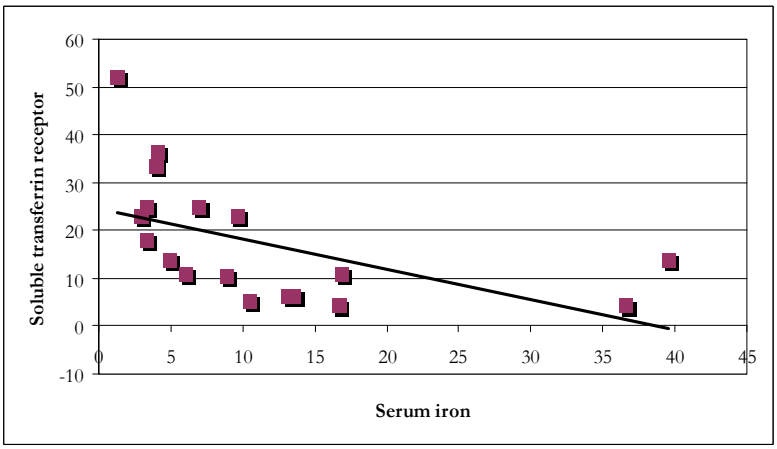

Figure 10.
Correlation between serum iron and the soluble transferrin receptor in the group of Kalafong patients with normal CRP (r-value = 0.52, p-value = 0.024).

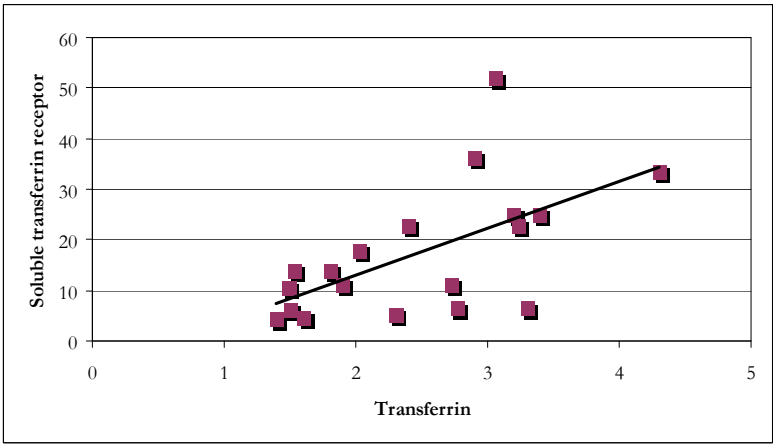

Figure 11.
Correlation between transferrin and the soluble transferrin receptor in the group of Kalafong patients with normal CRP (r-value = 0.59, p-value = 0.007).

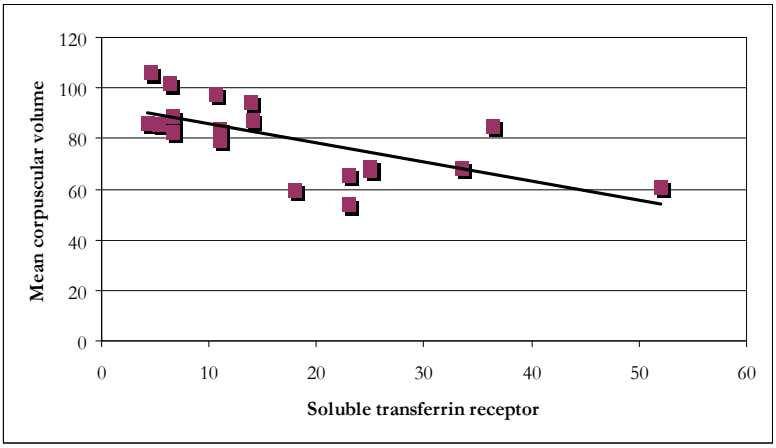

Figure 12.
Correlation between the soluble transferrin receptor and the mean corpuscular volume in the group of Kalafong patients with normal CRP (r-value = 0.65, p-value = 0.002).

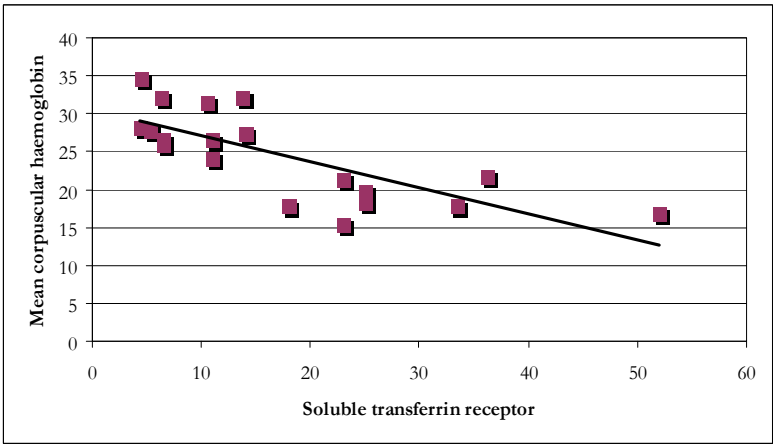

Figure 13.
Correlation between the soluble transferrin receptor and the mean corpuscular haemoglobin in the group of Kalafong patients with normal CRP (r-value = 0.75, p-value = 0.0002).

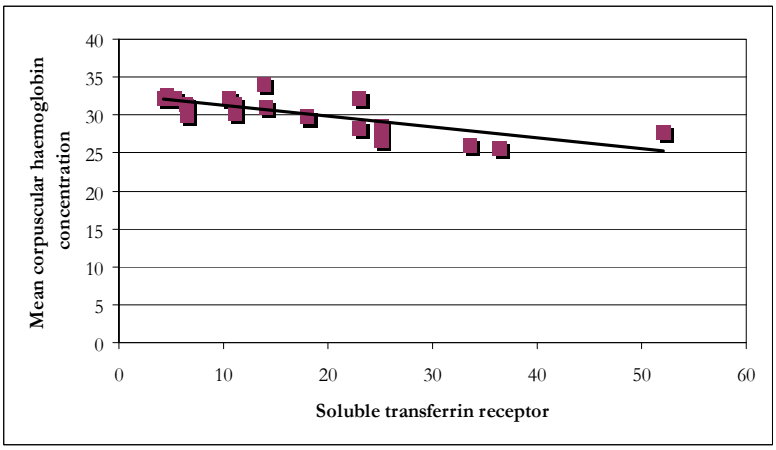

Figure 14.
Correlation between the soluble transferrin receptor and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal CRP (r-value = 0.75, p-value = 0.0002).

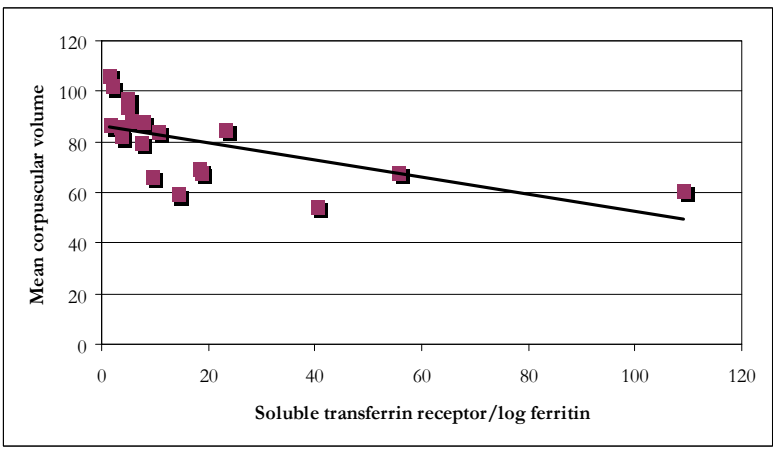

Figure 15.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular volume in the group of Kalafong patients with normal CRP (r-value = 0.60, p-value = 0.007).

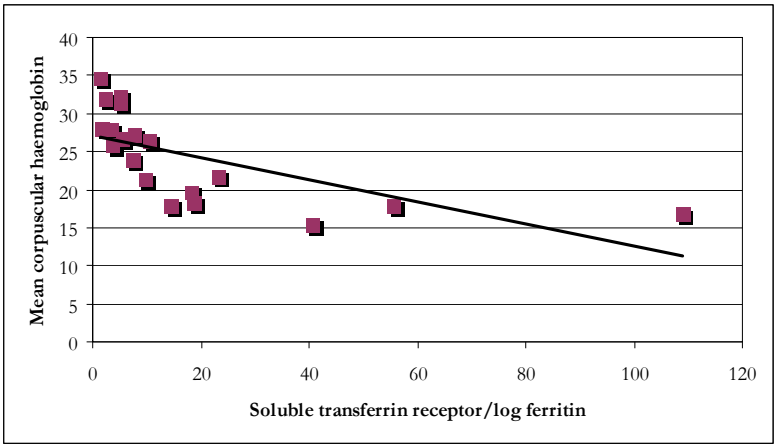

Figure 16.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular haemoglobin in the group of Kalafong patients with normal CRP (r-value = 0.65, p-value = 0.003).

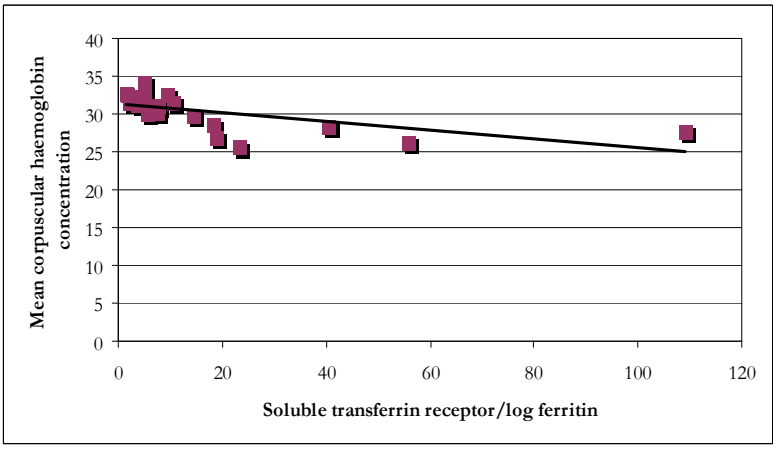

Figure 17.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal CRP (r-value = 0.61, p-value = 0.005).

5.2) Correlations in the group of Kalafong patients with normal neopterin

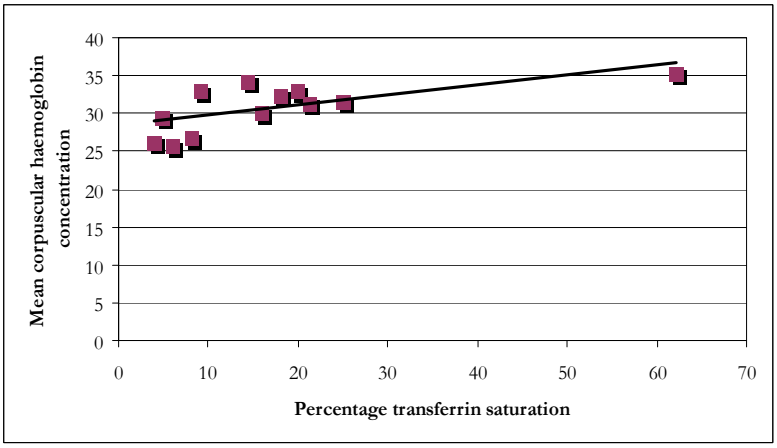

Figure 1.
Correlation between the percentage transferrin saturation and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.65, p-value = 0.015).

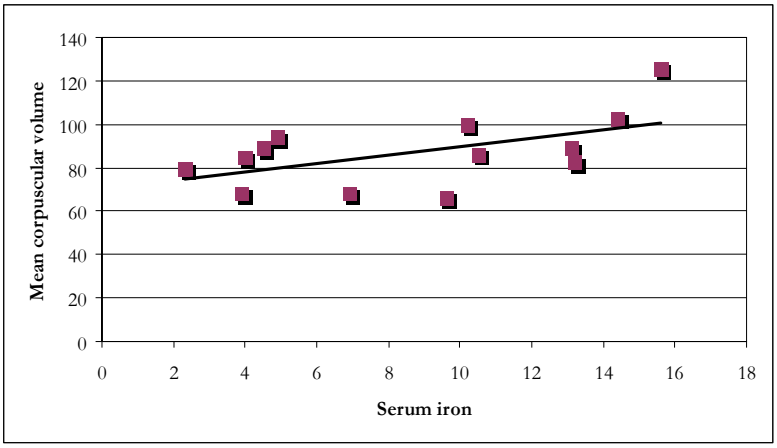

Figure 2.
Correlation between serum iron and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.55, p-value = 0.053).

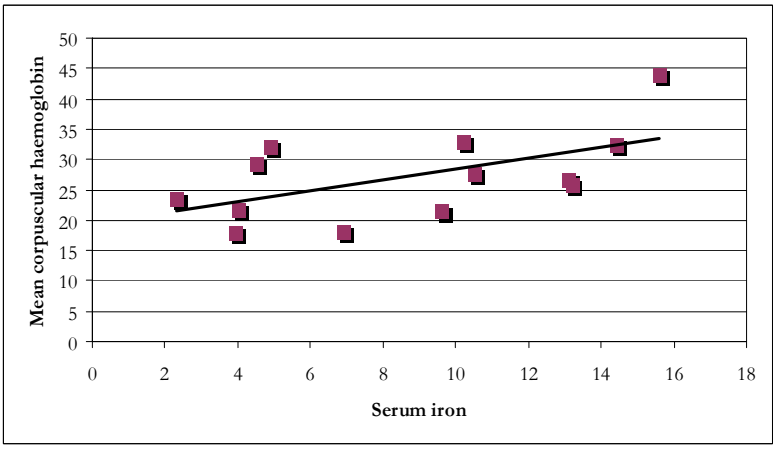

Figure 3.
Correlation between serum iron and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.57, p-value = 0.040).

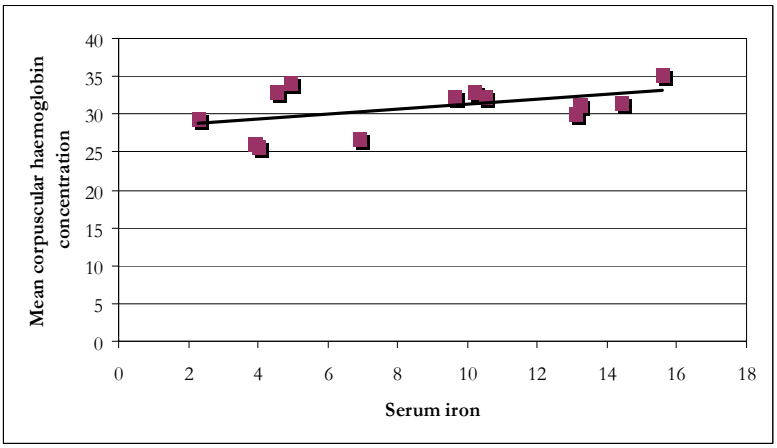

Figure 4.
Correlation between serum iron and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.49, p-value = 0.091).

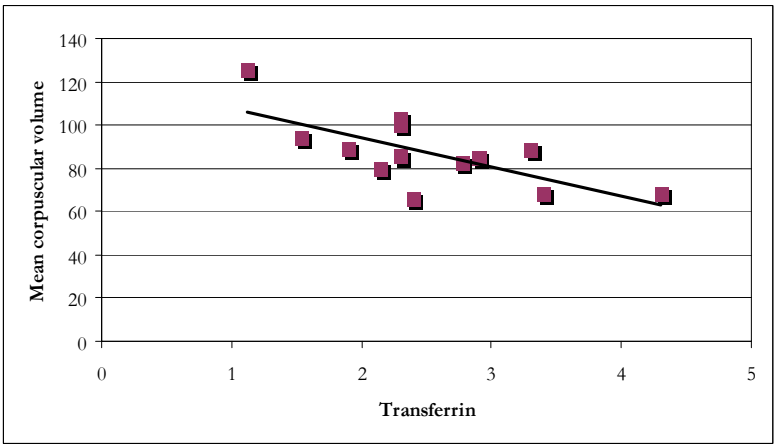

Figure 5.
Correlation between transferrin and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.70, p-value = 0.008).

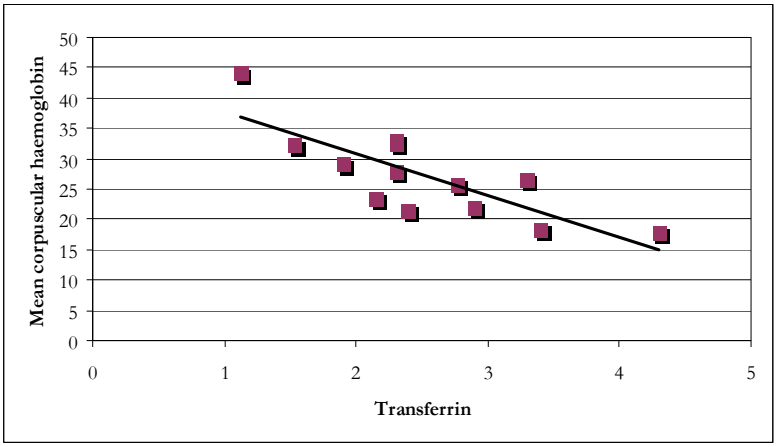

Figure 6.
Correlation between transferrin and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.80, p-value = 0.001).

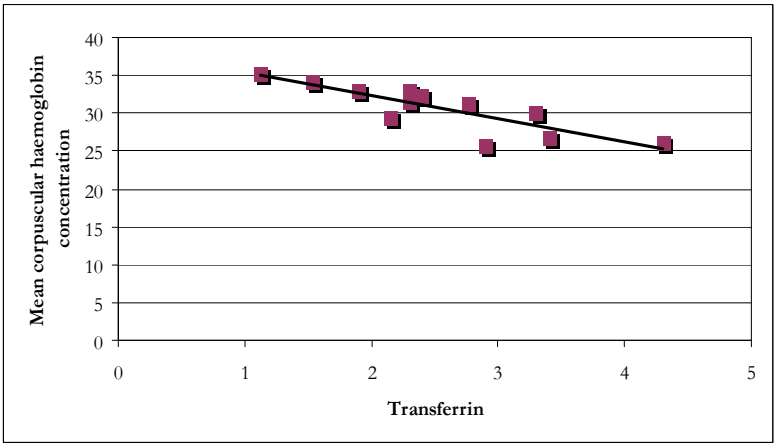

Figure 7.
Correlation between transferrin and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.84, p-value = 0.0003).

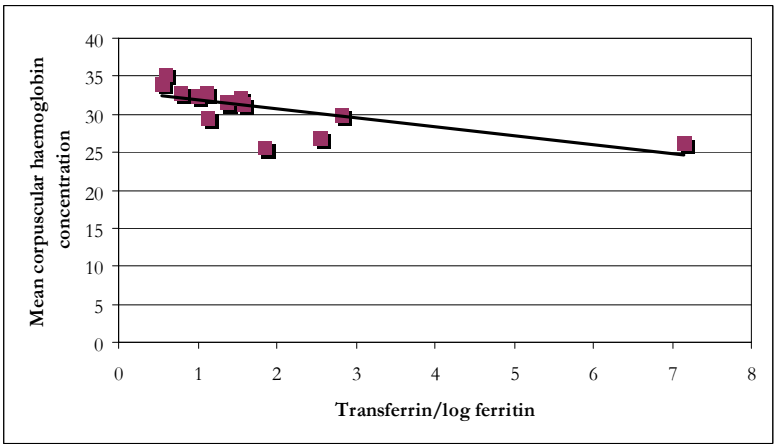

Figure 8.
Correlation between the transferrin/log ferritin ratio and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.68, p-value = 0.01).

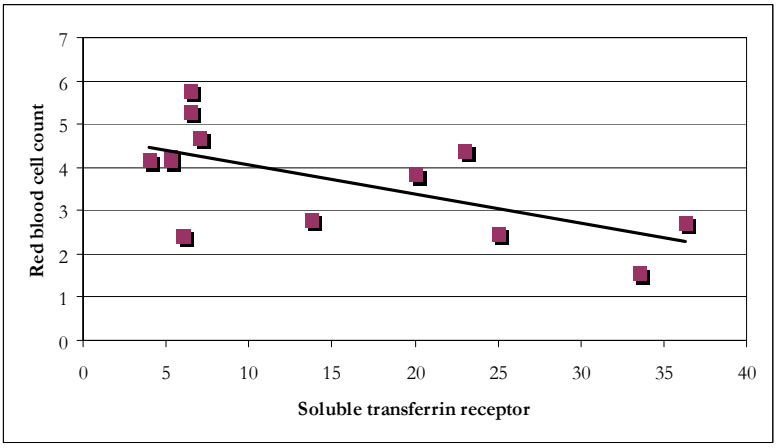

Figure 9.
Correlation between the soluble transferrin receptor and the red blood cell count in the group of Kalafong patients with normal neopterin (r-value = 0.63, p-value = 0.021).

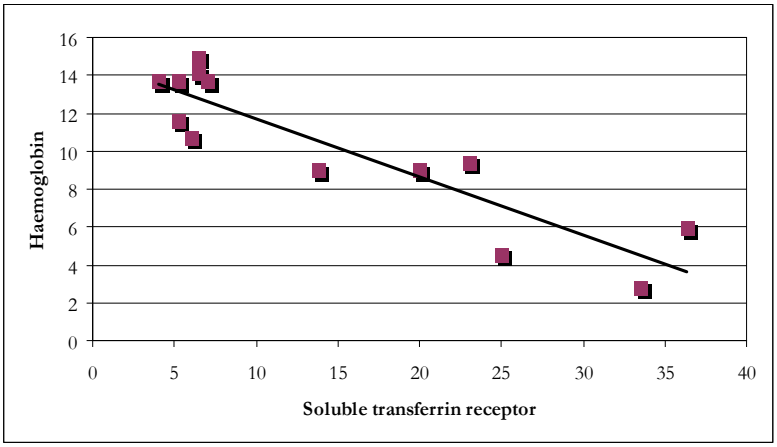

Figure 10.
Correlation between the soluble transferrin receptor and haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.90, p-value < 0.0001).

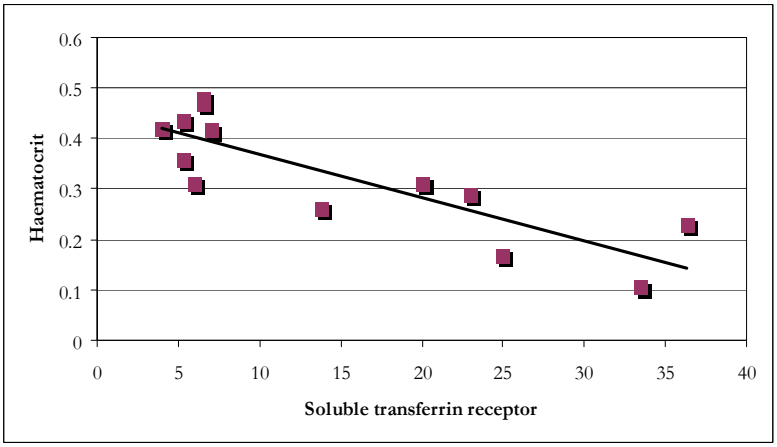

Figure 11.
Correlation between the soluble transferrin receptor and haematocrit in the group of Kalafong patients with normal neopterin (r-value = 0.84, p-value = 0.0003).

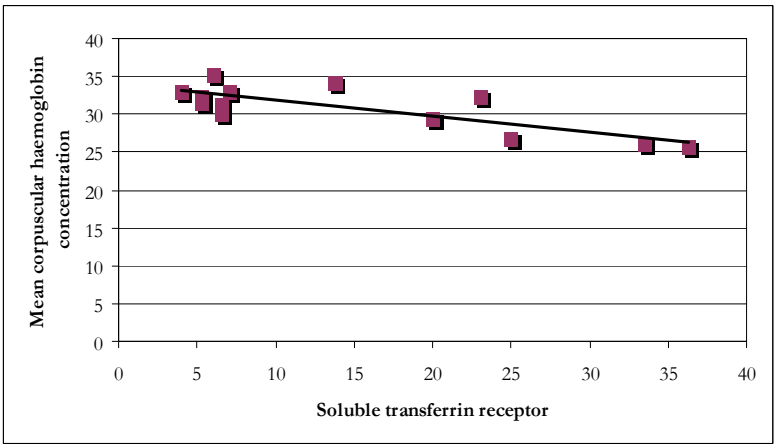

Figure 12.
Correlation between the soluble transferrin receptor and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.79, p-value = 0.001).

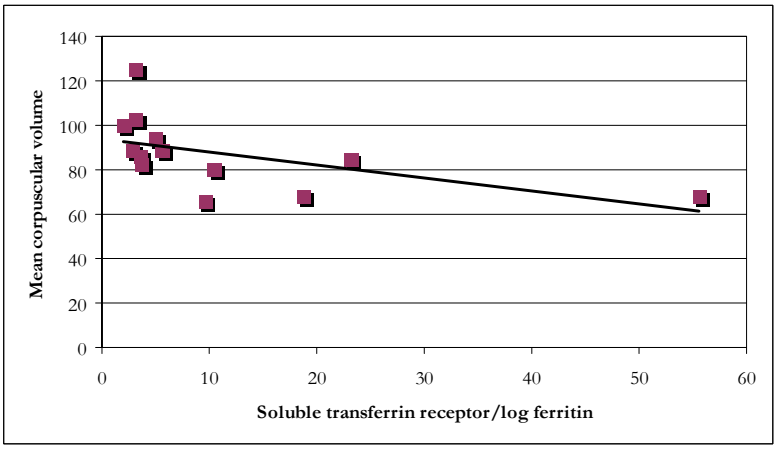

Figure 13.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.53, p-value = 0.06).

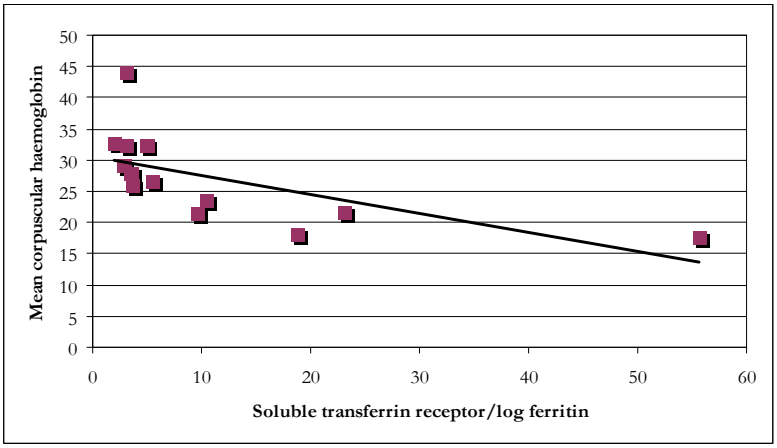

Figure 14.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.63, p-value = 0.021).

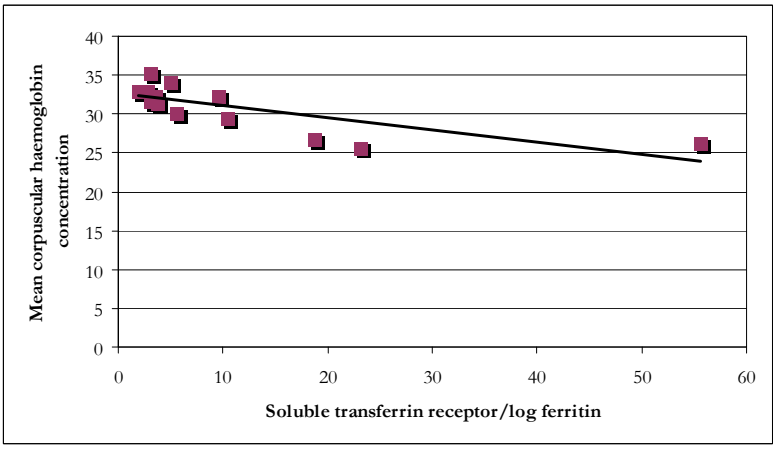

Figure 15.
Correlation between the soluble transferrin receptor/log ferritin ratio and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with normal neopterin (r-value = 0.75, p-value = 0.003).

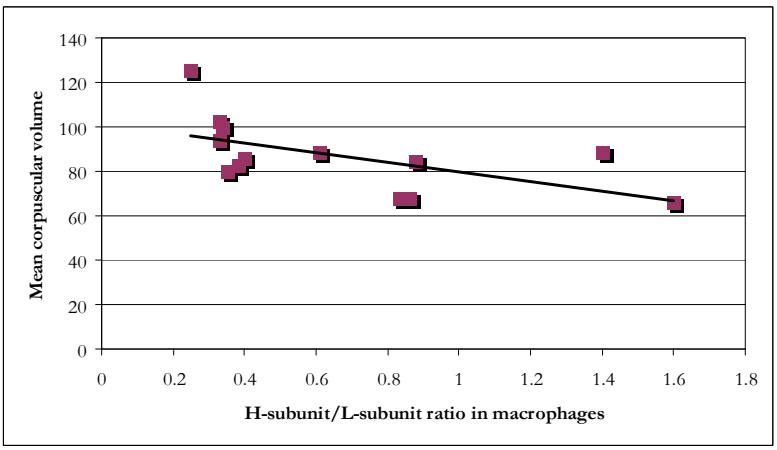

Figure 16.
Correlation between the H-subunit/L-subunit ratio in macrophages and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.58, p-value = 0.037).

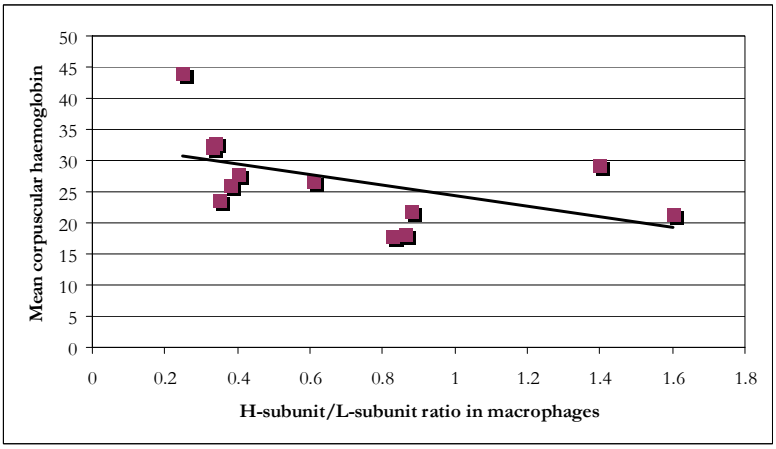

Figure 17.
Correlation between the H-subunit/L-subunit ratio in macrophages and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.51, p-value = 0.072).

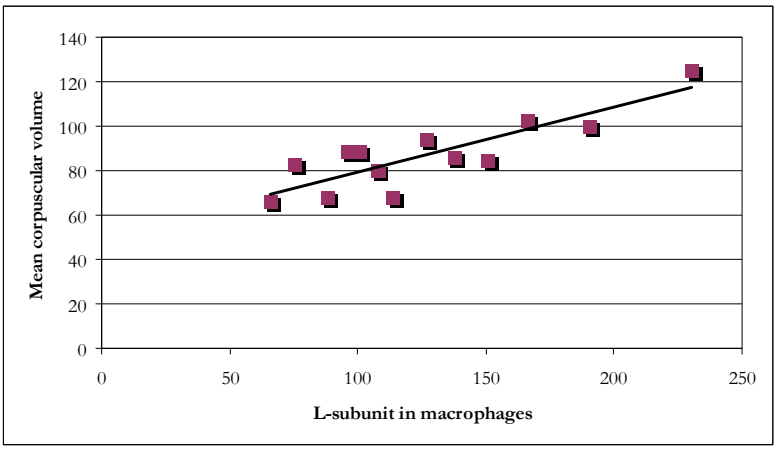

Figure 18.
Correlation between the L-subunit in macrophages and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.85, p-value = 0.0002).

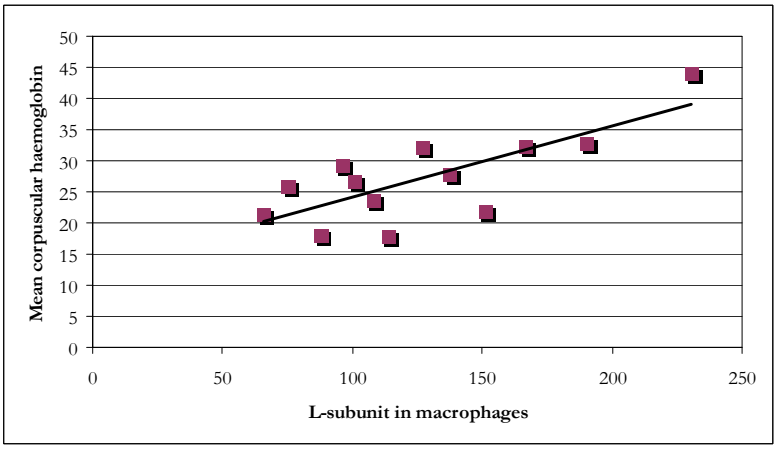

Figure 19.
Correlation between the L-subunit in macrophages and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.76, p-value = 0.003).

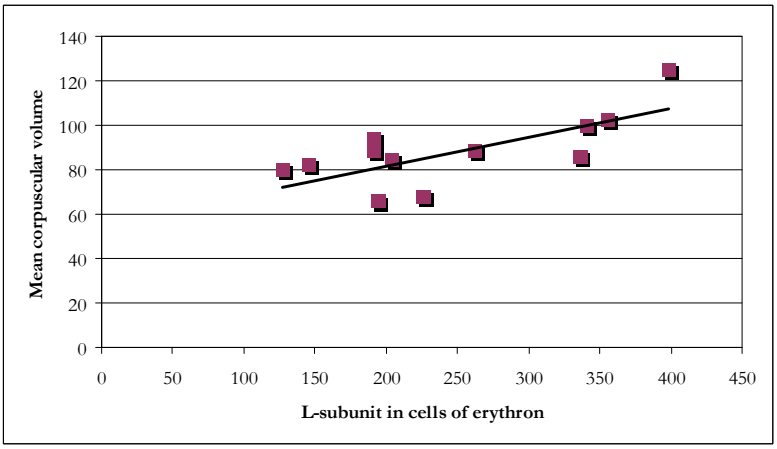

Figure 20.
Correlation between the L-subunit in cells of the erythron and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.69, p-value = 0.01).

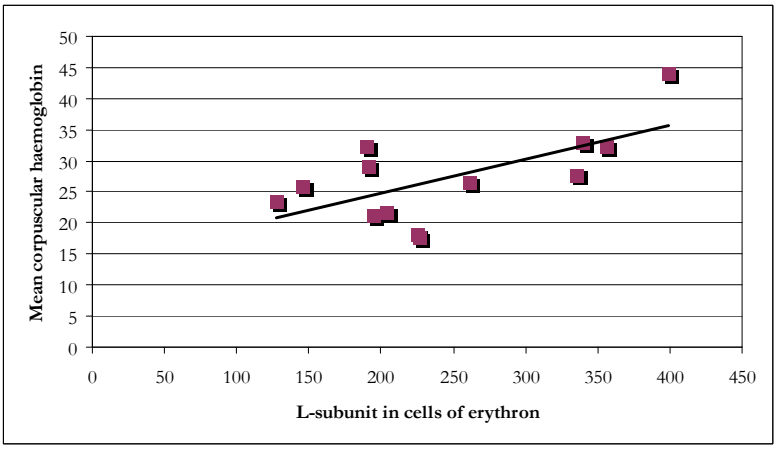

Figure 21.
Correlation between the L-subunit in cells of the erythron and the mean corpuscular haemoglobin in the group of Kalafong patients with normal neopterin (r-value = 0.65, p-value = 0.016).

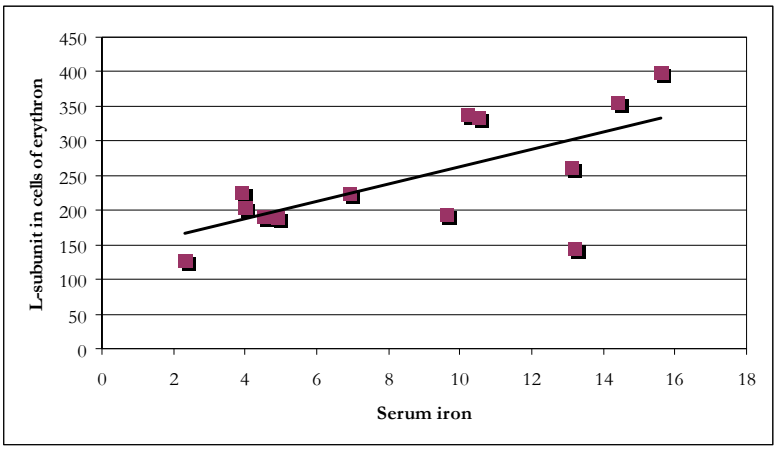

Figure 22.
Correlation between serum iron and the L-subunit in cells of the erythron in the group of Kalafong patients with normal neopterin (r-value = 0.66, p-value = 0.014).

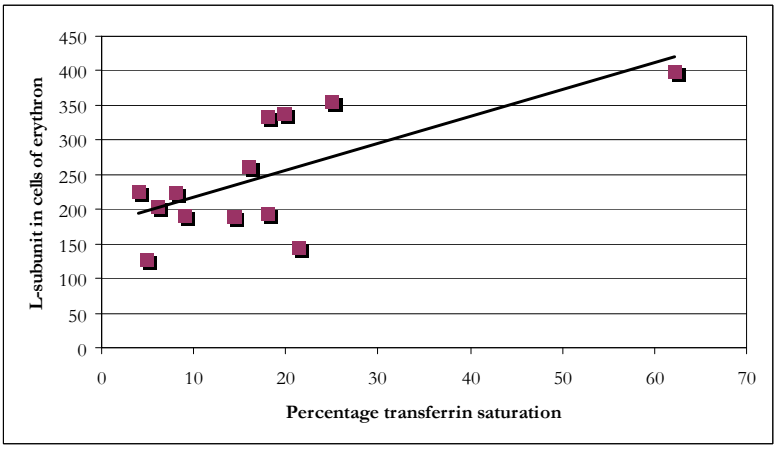

Figure 23.
Correlation between percentage transferrin saturation and the L-subunit in cells of the erythron in the group of Kalafong patients with normal neopterin (r-value = 0.68, p-value = 0.01).

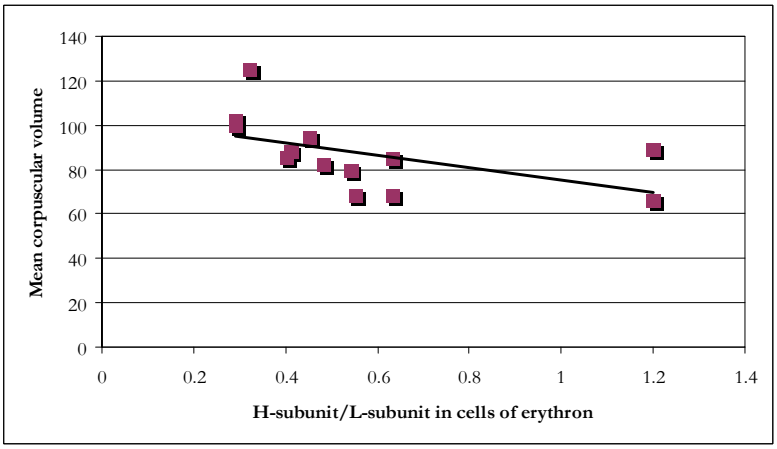

Figure 24.
Correlation between the H-subunit/L-subunit ratio in cells of the erythron and the mean corpuscular volume in the group of Kalafong patients with normal neopterin (r-value = 0.52, p-value = 0.069).

5.3) Correlations in the group of Kalafong patients with no iron transfer block

Figure 1.
Correlation between serum iron and the mean corpuscular volume in the group of Kalafong patients with no iron transfer block (r-value = 0.57, p-value = 0.004).

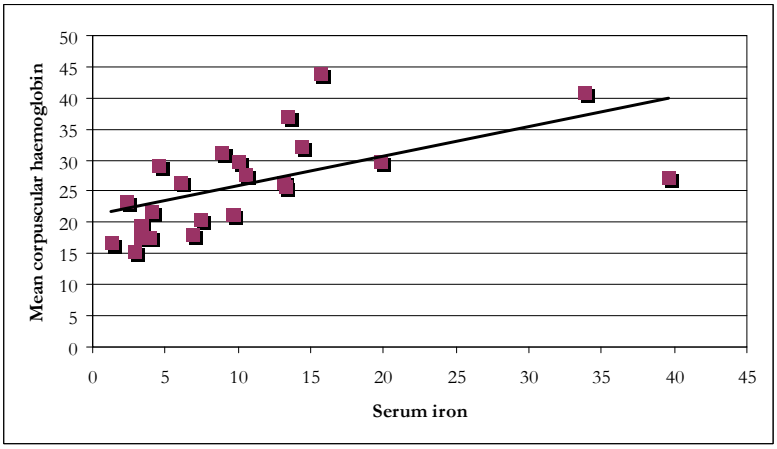

Figure 2.
Correlation between serum iron and the mean corpuscular haemoglobin in the group of Kalafong patients with no iron transfer block (r-value = 0.59, p-value = 0.003).

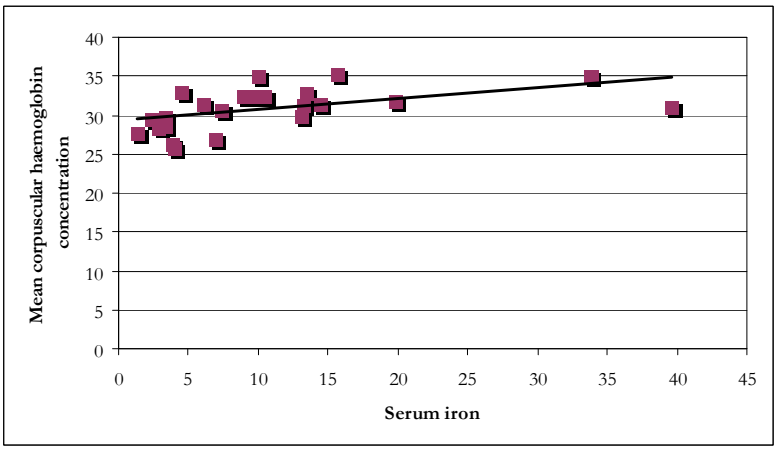

Figure 3.
Correlation between serum iron and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with no iron transfer block (r-value = 0.50, p-value = 0.016).

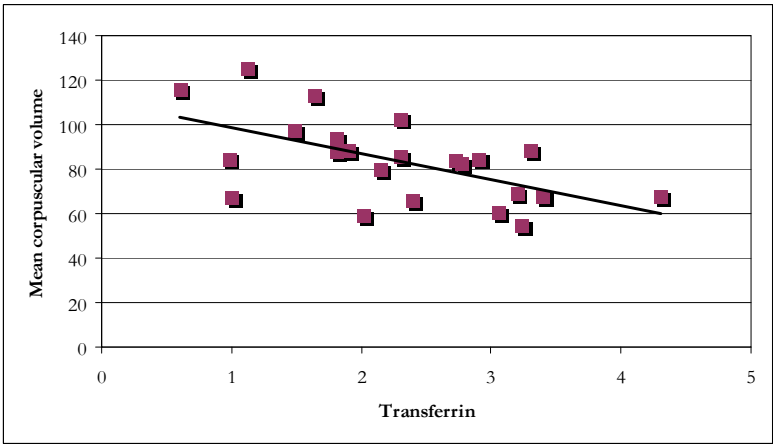

Figure 4.
Correlation between transferrin and the mean corpuscular volume in the group of Kalafong patients with no iron transfer block (r-value = 0.58, p-value = 0.004).

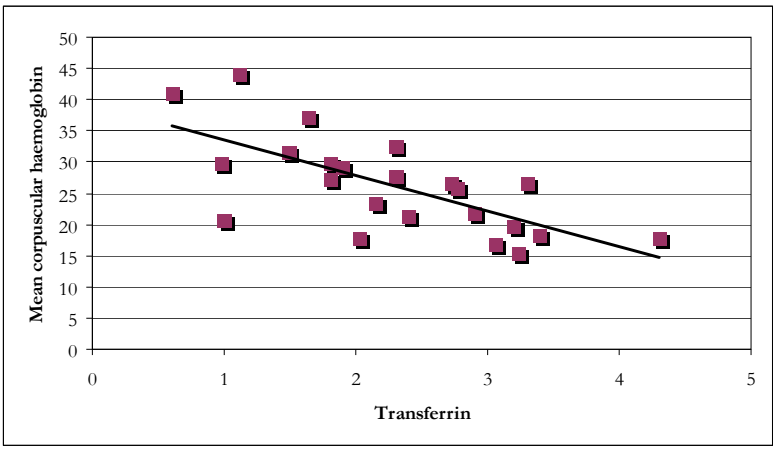

Figure 5.
Correlation between transferrin and the mean corpuscular haemoglobin in the group of Kalafong patients with no iron transfer block (r-value = 0.69, p-value = 0.0003).

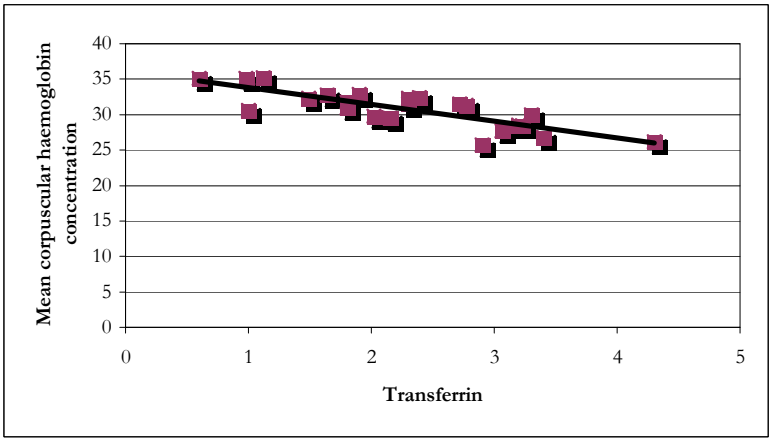

Figure 6.
Correlation between transferrin and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with no iron transfer block (r-value = 0.82, p-value < 0.0001).

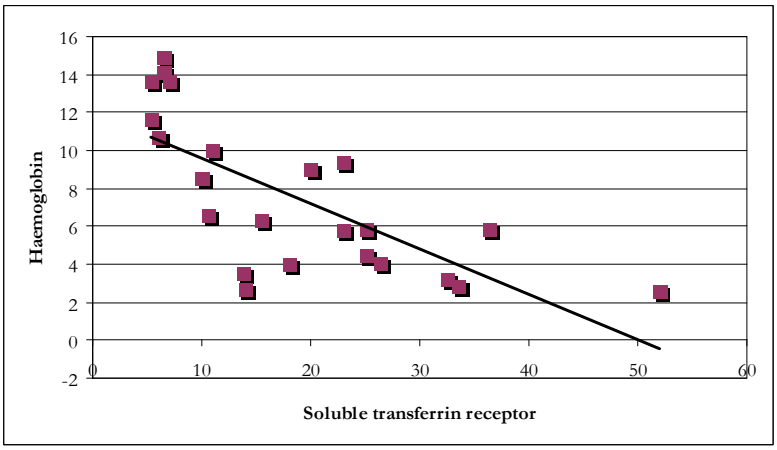

Figure 7.
Correlation between the soluble transferrin receptor and haemoglobin in the group of Kalafong patients with no iron transfer block (r-value = 0.71, p-value = 0.0001).

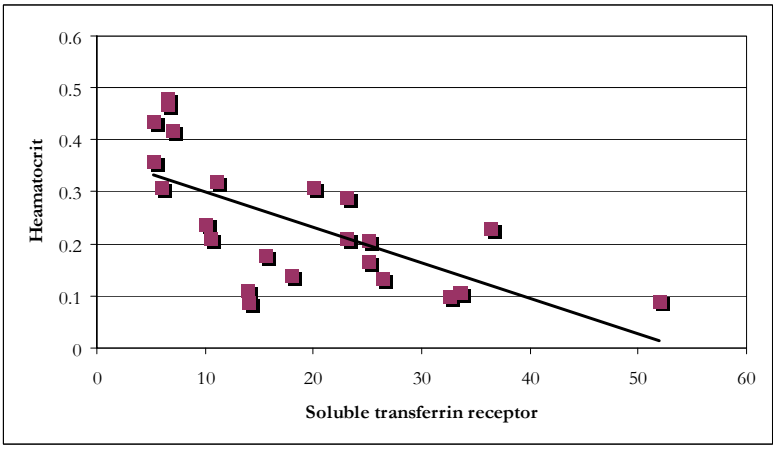

Figure 8.
Correlation between the soluble transferrin receptor and haematocrit in the group of Kalafong patients with no iron transfer block (r-value = 0.66, p-value = 0.0006).

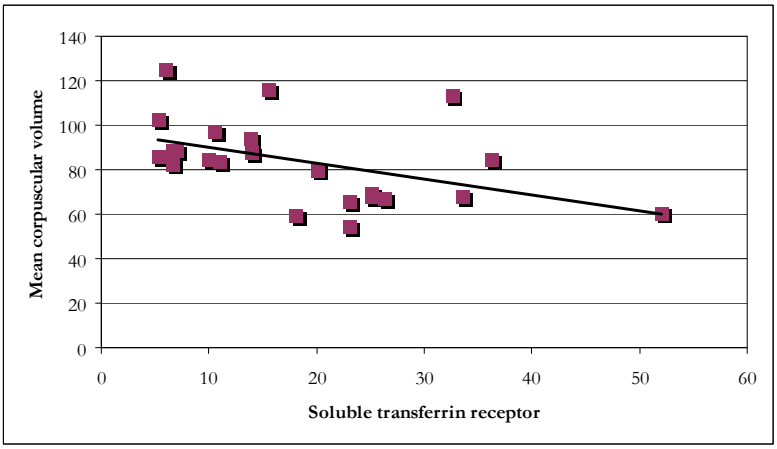

Figure 9.
Correlation between the soluble transferrin receptor and the mean corpuscular volume in the group of Kalafong patients with no iron transfer block (r-value = 0.46, p-value = 0.027).

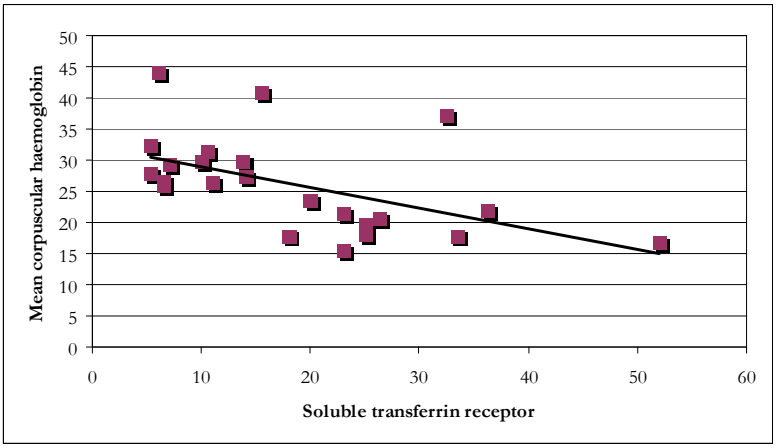

Figure 10.
Correlation between the soluble transferrin receptor and the mean corpuscular haemoglobin in the group of Kalafong patients with no iron transfer block (r-value = 0.53, p-value = 0.01).

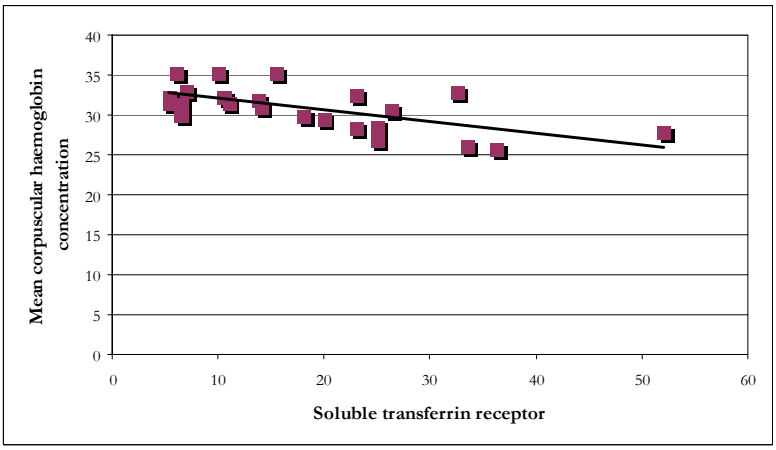

Figure 11.
Correlation between the soluble transferrin receptor and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with no iron transfer block (r-value = 0.64, p-value = 0.001).

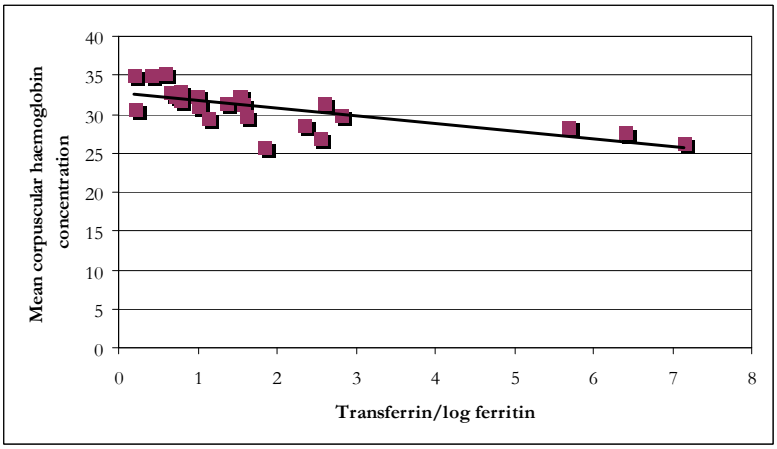

Figure 12.
Correlation between the transferrin/log ferritin ratio and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with no iron transfer block (r-value = 0.69, p-value = 0.0003).

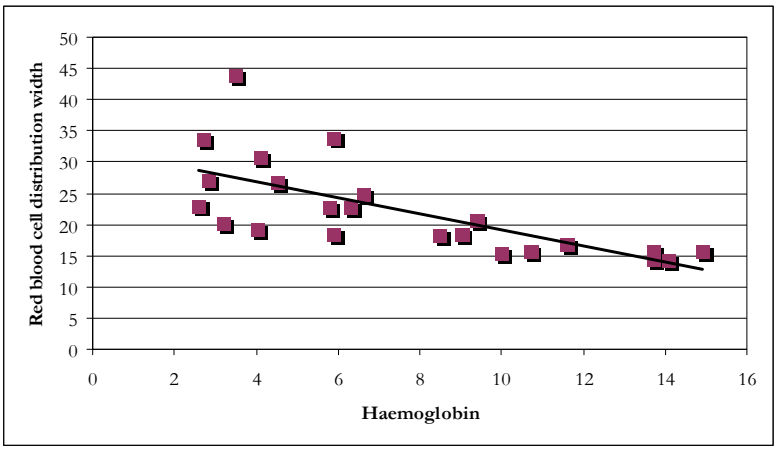

Figure 13.
Correlation between haemoglobin and the red blood cell distribution width in the group of Kalafong patients with no iron transfer block (r-value = 0.70, p-value = 0.0002).

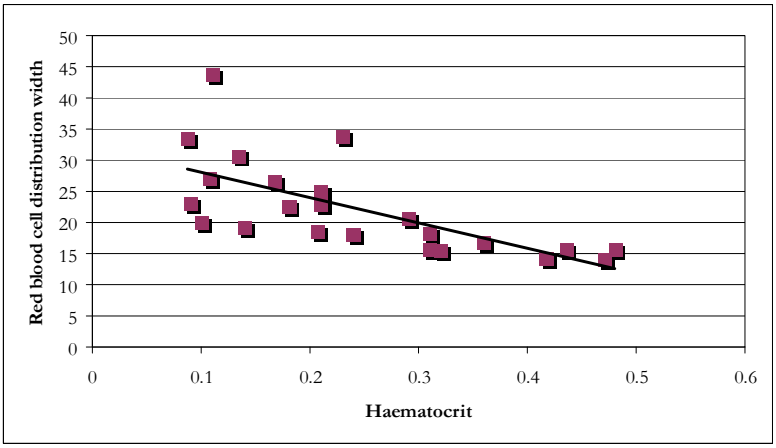

Figure 14.
Correlation between haematocrit and the red blood cell distribution width in the group of Kalafong patients with no iron transfer block (r-value = 0.68, p-value = 0.0003).

5.4) Correlations in the group of Kalafong patients with high C-reactive protein

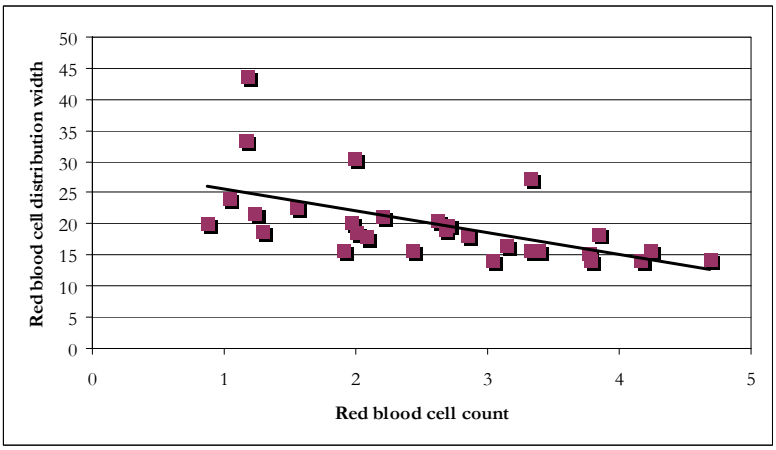

Figure 1.
Correlation between the red blood cell count and the red blood cell distribution width in the group of Kalafong patients with high CRP (r-value = 0.57, p-value = 0.001).

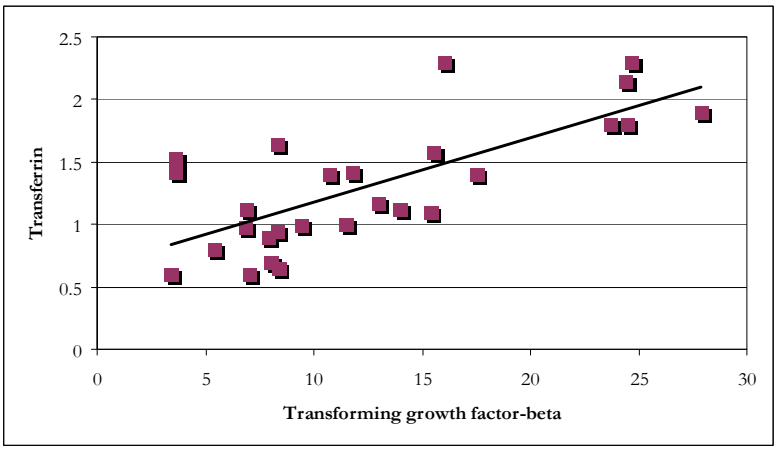

Figure 2.
Correlation between transforming growth factor-beta and transferrin in the group of Kalafong patients with high CRP (r-value = 0.73, p-value < 0.0001).

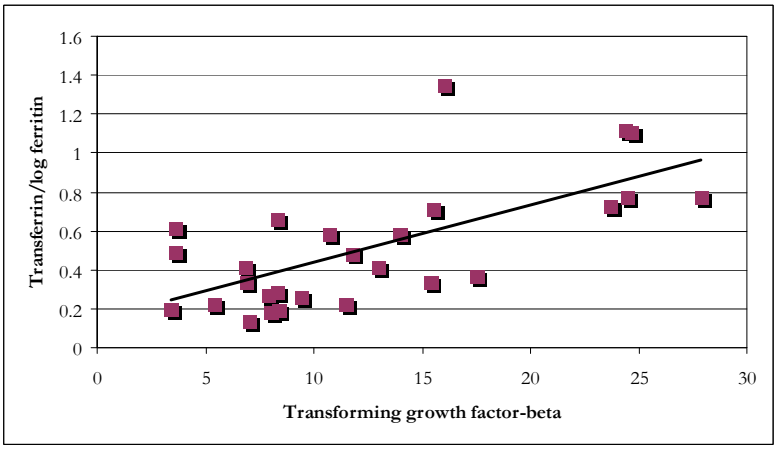

Figure 3.
Correlation between transforming growth factor-beta and the transferrin/log ferritin ratio in the group of Kalafong patients with high CRP (r-value = 0.67, p-value = 0.0001).

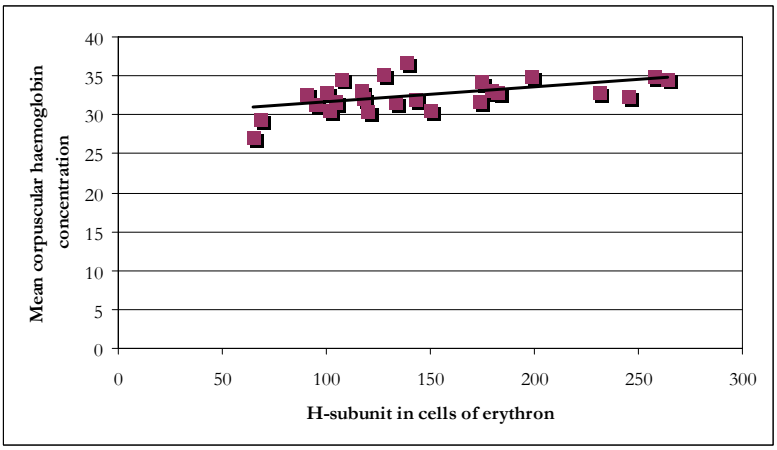

Figure 4.
Correlation between the H-subunit in cells of the erythron and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with high CRP (r-value = 0.51, p-value = 0.007).

5.5) Correlations in the group of Kalafong patients with high neopterin

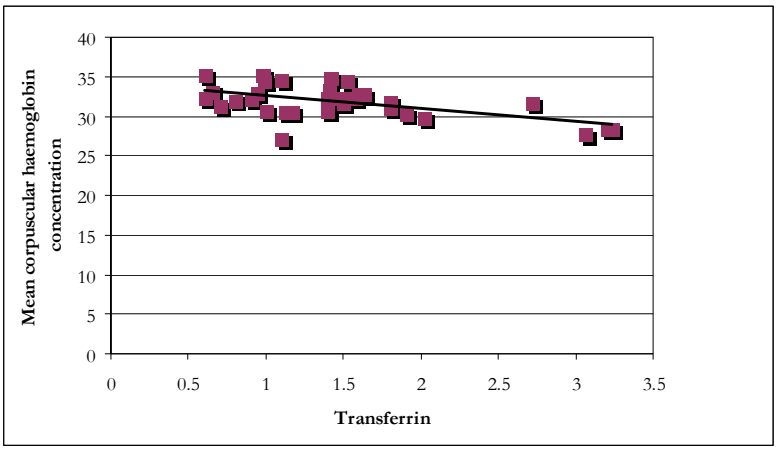

Figure 1.
Correlation between transferrin and the mean corpuscular haemoglobin concentration in the group of Kalafong patients with high neopterin (r-value = 0.56, p-value = 0.0006).

5.6) Correlations in the group of Kalafong patients with iron transfer block

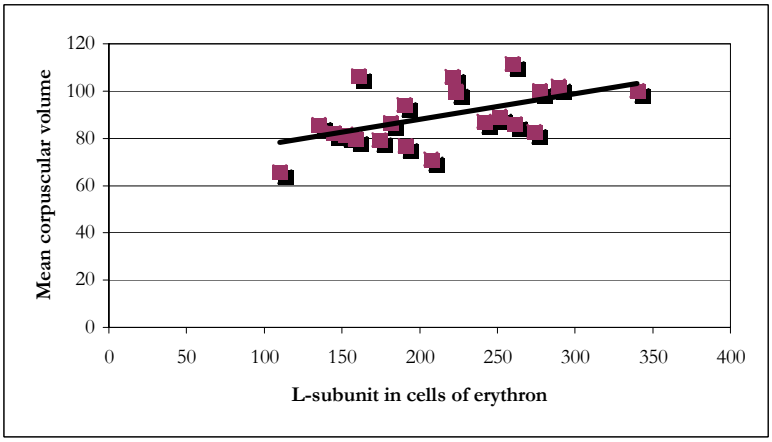

Figure 1.
Correlation between the L-subunit in cells of the erythron and the mean corpuscular volume in the group of Kalafong patients with iron transfer block (r-value = 0.53, p-value = 0.014).

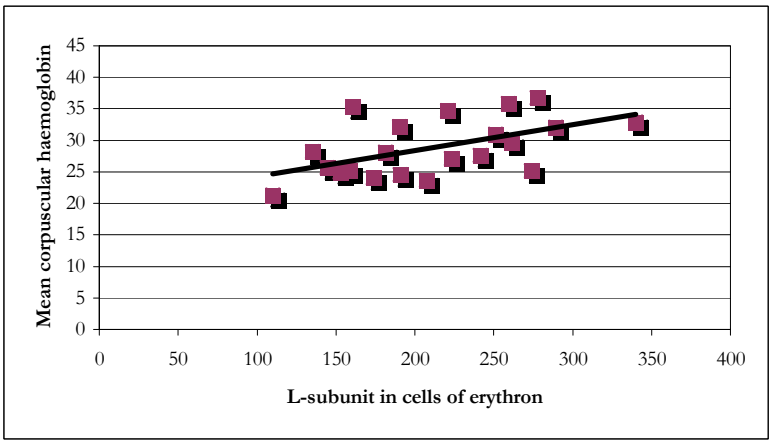

Figure 2.
Correlation between the L-subunit in cells of the erythron and the mean corpuscular haemoglobin in the group of Kalafong patients with iron transfer block (r-value = 0.53, p-value = 0.013).

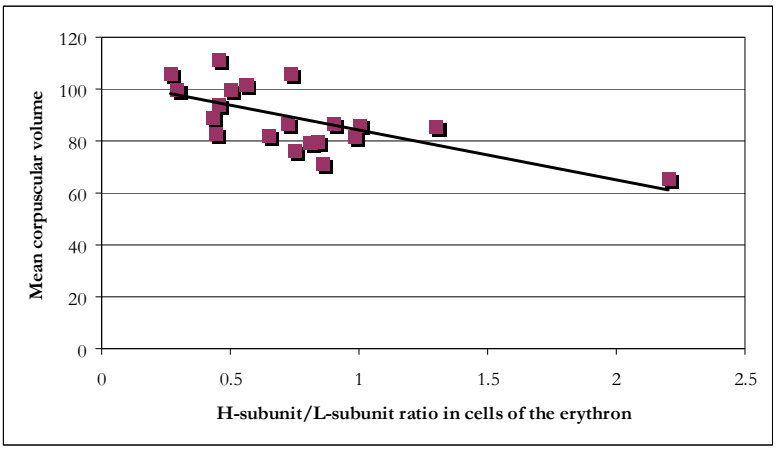

Figure 3.
Correlation between the H-subunit/L-subunit ratio in cells of the erythron and the mean corpuscular volume in the group of Kalafong patients with iron transfer block (r-value = 0.55, p-value = 0.008).

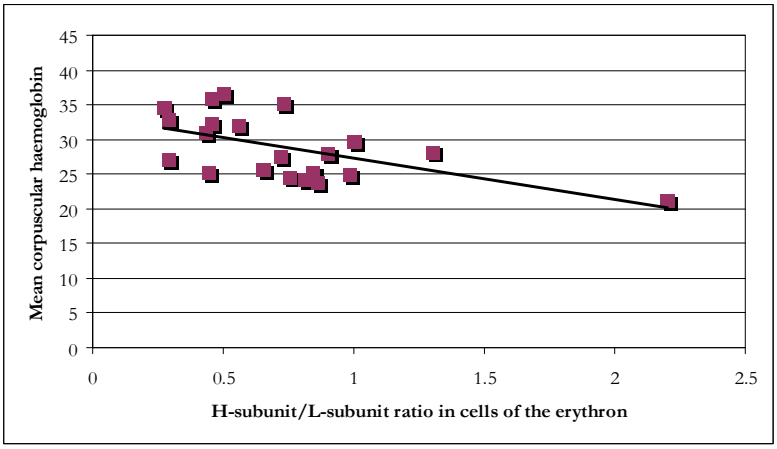

Figure 4.
Correlation between the H-subunit/L-subunit ratio in cells of the erythron and the mean corpuscular haemoglobin in the group of Kalafong patients with iron transfer block (r-value = 0.42, p-value = 0.049).

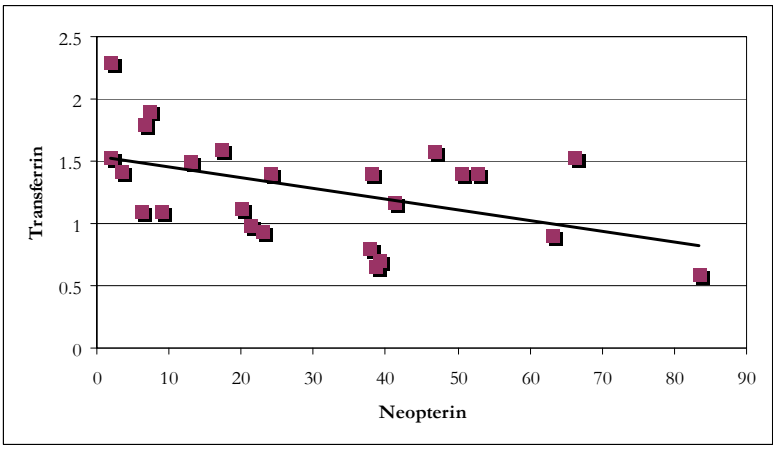

Figure 5.
Correlation between neopterin and transferrin in the group of Kalafong patients with iron transfer block (r-value = 0.47, p-value = 0.019).

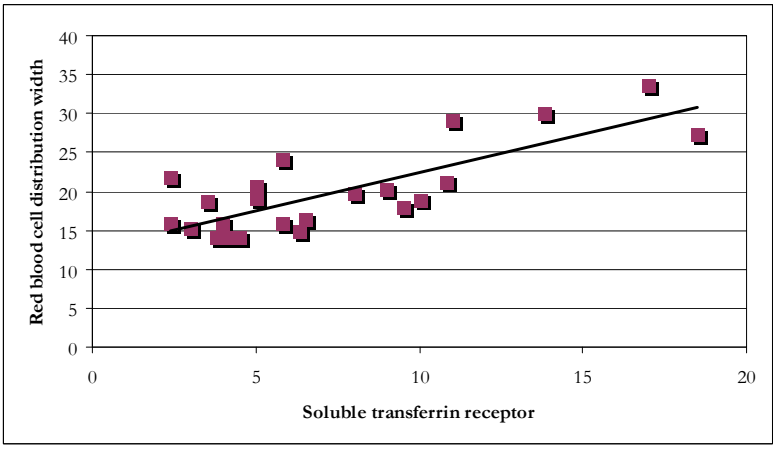

Figure 6.
Correlation between the soluble transferrin receptor and the red blood cell distribution width in the group of Kalafong patients with iron transfer block (r-value = 0.79, p-value < 0.0001).

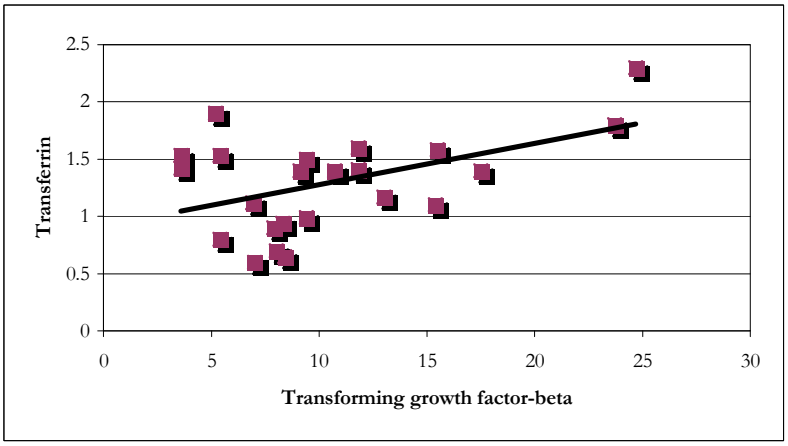

Figure 7.
Correlation between transforming growth factor-beta and transferrin in the group of Kalafong patients with iron transfer block (r-value = 0.48, p-value = 0.02).

5.7) Correlations in the group of osteoarthritis patients

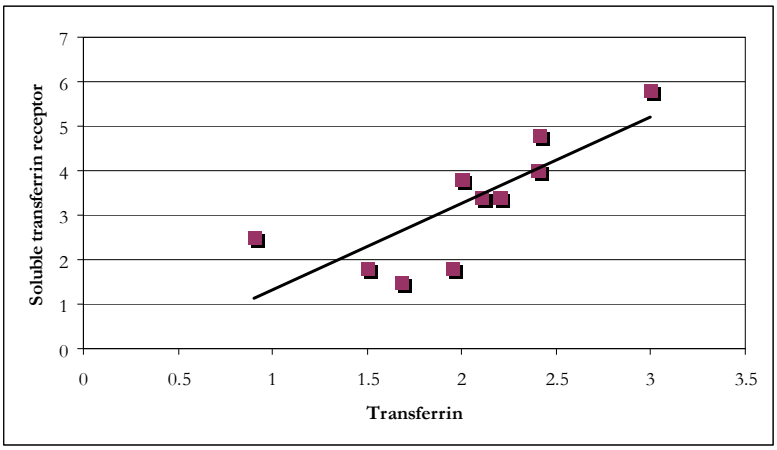

Figure 1.
Correlation between transferrin and the soluble transferrin receptor in the group of osteoarthritis patients (r-value = 0.79, p-value = 0.006).

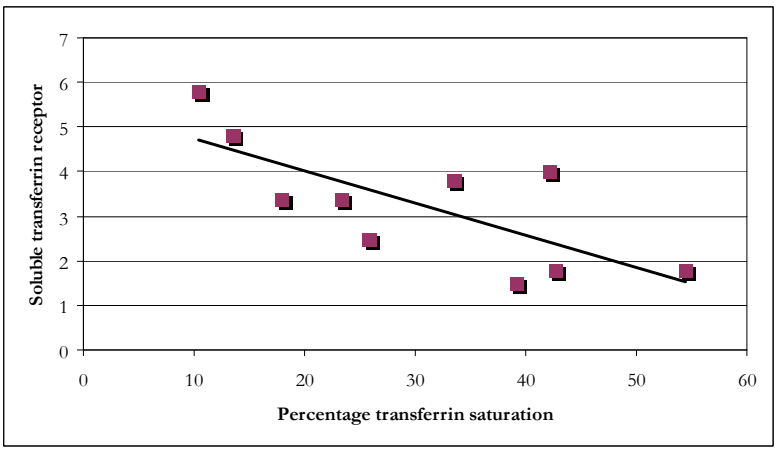

Figure 2.
Correlation between the percentage transferrin saturation and the soluble transferrin receptor in the group of osteoarthritis patients (r-value = 0.74, p-value = 0.014).

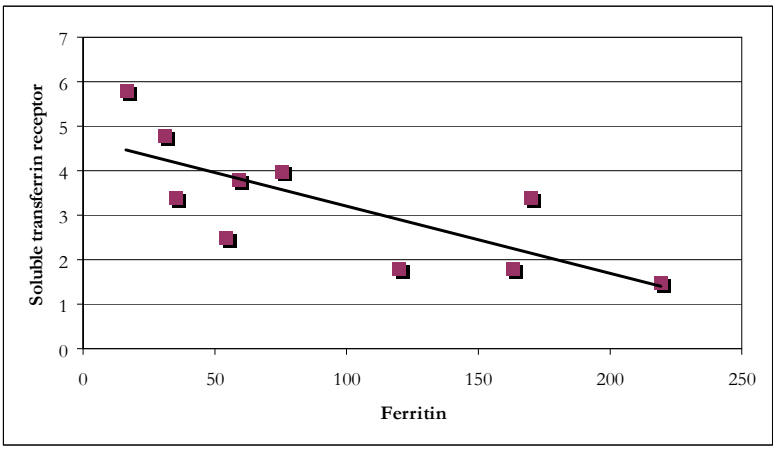

Figure 3.
Correlation between ferritin and the soluble transferrin receptor in the group of osteoarthritis patients (r-value = 0.75, p-value = 0.013).

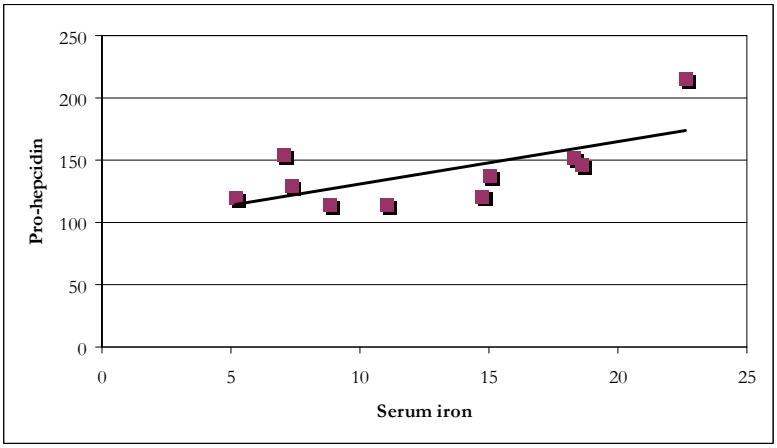

Figure 4.
Correlation between serum iron and pro-hepcidin in the group of osteoarthritis patients (r-value = 0.67, p-value = 0.034).

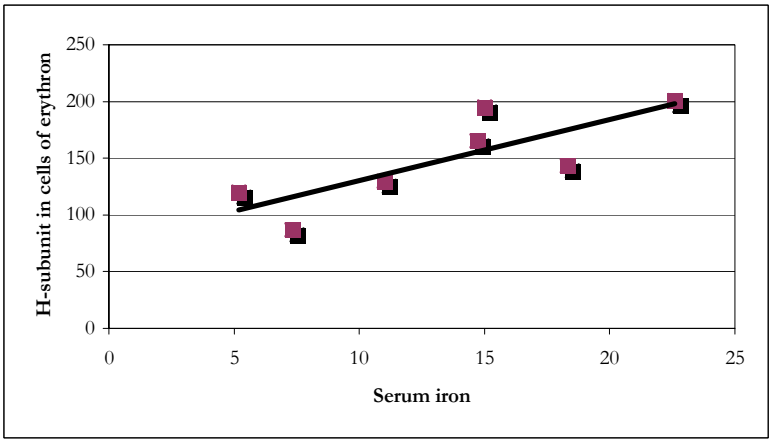

Figure 5.
Correlation between serum iron and the H-subunit in cells of the erythron in the group of osteoarthritis patients (r-value = 0.80, p-value = 0.031).

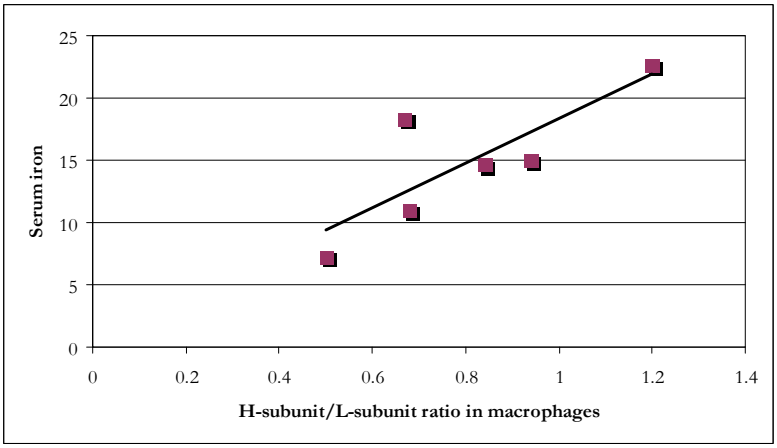

Figure 6.
Correlation between the H-subunit/L-subunit ratio in macrophages and serum iron in the group of osteoarthritis patients (r-value = 0.82, p-value = 0.047).

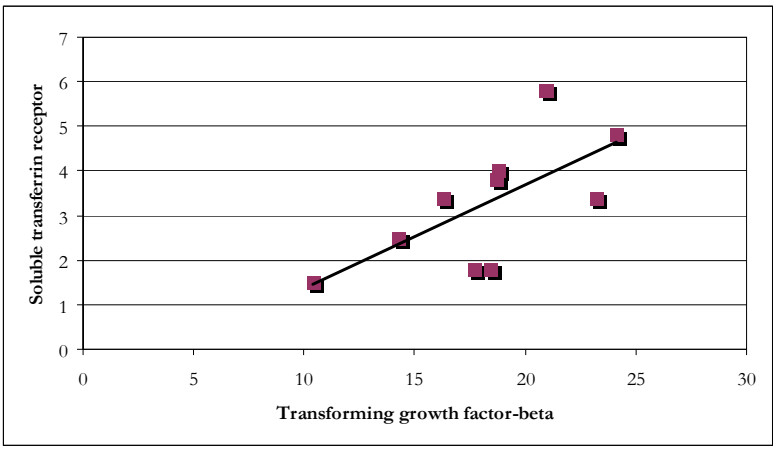

Figure 7.
Correlation between transforming growth factor-beta and the soluble transferrin receptor in the group of osteoarthritis patients (r-value = 0.67, p-value = 0.034).

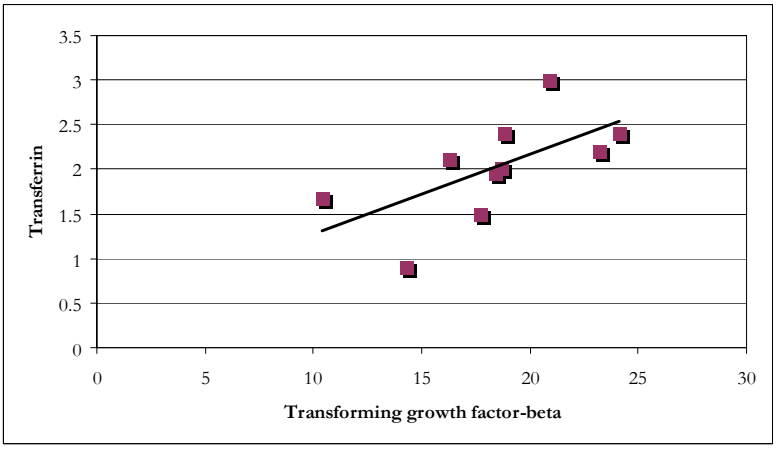

Figure 8.
Correlation between transforming growth factor-beta and transferrin in the group of osteoarthritis patients (r-value = 0.63, p-value = 0.05).

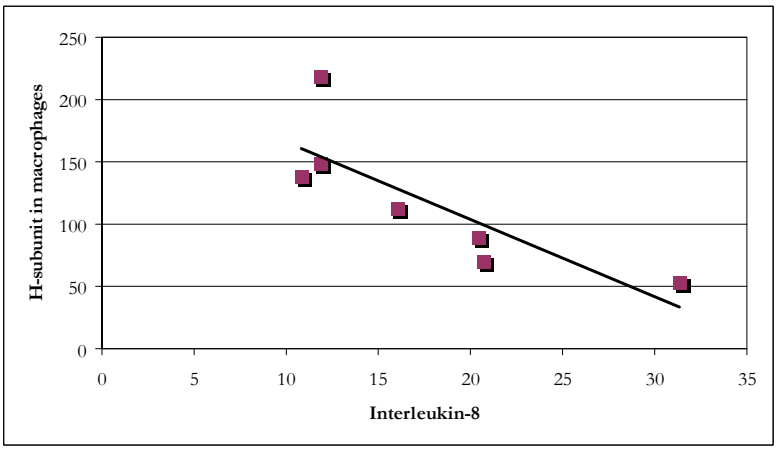

Figure 9.
Correlation between interleukin-8 and the H-subunit in macrophages in the group of osteoarthritis patients (r-value = 0.81, p-value = 0.026).

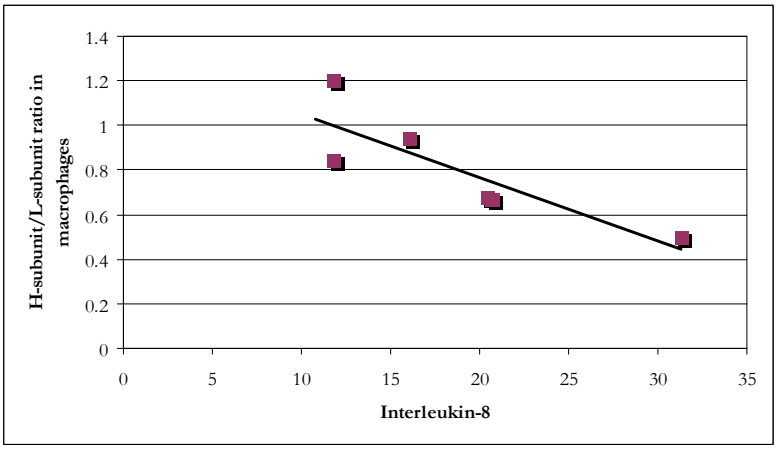

Figure 10.
Correlation between interleukin-8 and the H-subunit/L-subunit ratio in macrophages in the group of osteoarthritis patients (r-value = 0.84, p-value = 0.037).

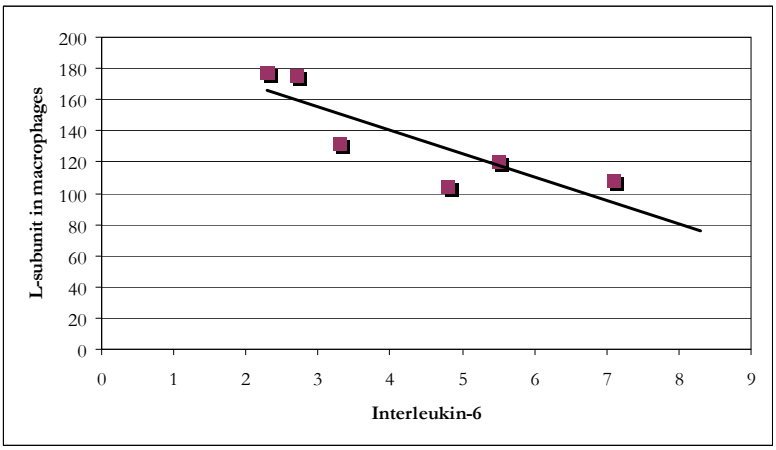

Figure 11.
Correlation between interleukin-6 and the L-subunit in macrophages in the group of osteoarthritis patients (r-value = 0.85, p-value = 0.034).

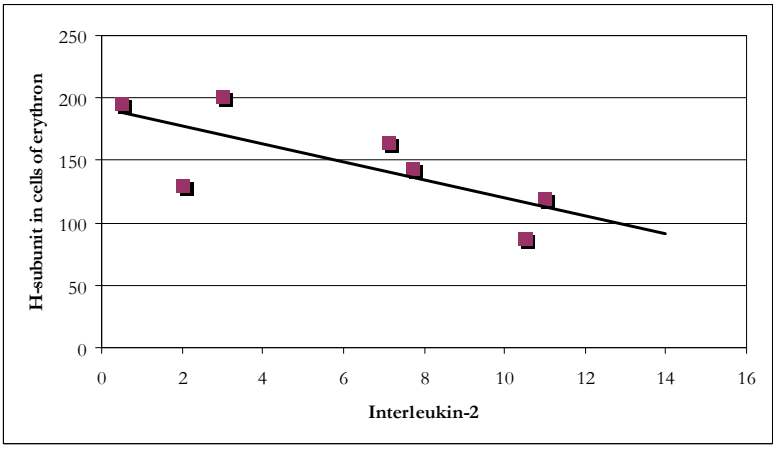

Figure 12.
Correlation between interleukin-2 and the H-subunit in cells of the erythron in the group of osteoarthritis patients (r-value = 0.73, p-value = 0.064).

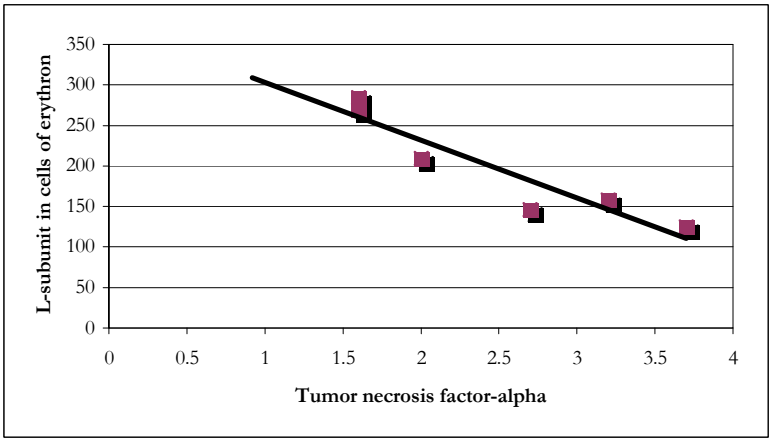

Figure 13.
Correlation between tumor necrosis factor-alpha and the L-subunit in cells of the erythron in the group of osteoarthritis patients (r-value = 0.94, p-value = 0.006).

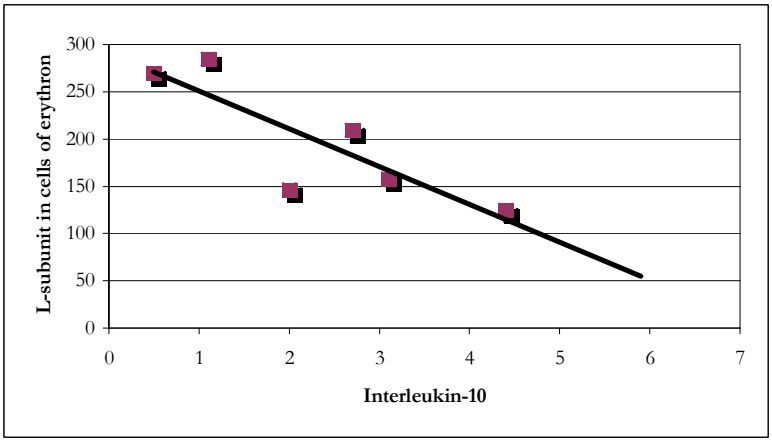

Figure 14.
Correlation between interleukin-10 and the L-subunit in cells of the erythron in the group of osteoarthritis patients (r-value = 0.85, p-value = 0.03).

Electron micrographs and raw data of immunolabelling of H-subunit and L-subunit of ferritin, photographs of the Prussian blue iron stains and the presence or absence of an iron transfer block are contained in volume 2, chapter 6.