Can we detect bone cancer in the blood?
Biomarkers of clinical outcomes in osteosarcoma

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Not All Cancer Is Pink

Bone Cancer Awareness

Introduction

Osteosarcoma is the most common type of primary malignant bone tumour, which is a type of cancer that arises from bone, occurring mainly in adolescents and the elderly[1]. There is a great need to identify osteosarcoma patients' response to standard chemotherapy and to provide alternative treatments. This has led to a strong interest in the identification of biomarkers (biological measurable indicators) in the blood that correspond with outcome in osteosarcoma[2]. Newcastle upon Tyne Hospitals has a combined clinical and research focus on primary bone tumours in North England. The aim was to use this opportunity to explore biomarker detection in osteosarcoma.
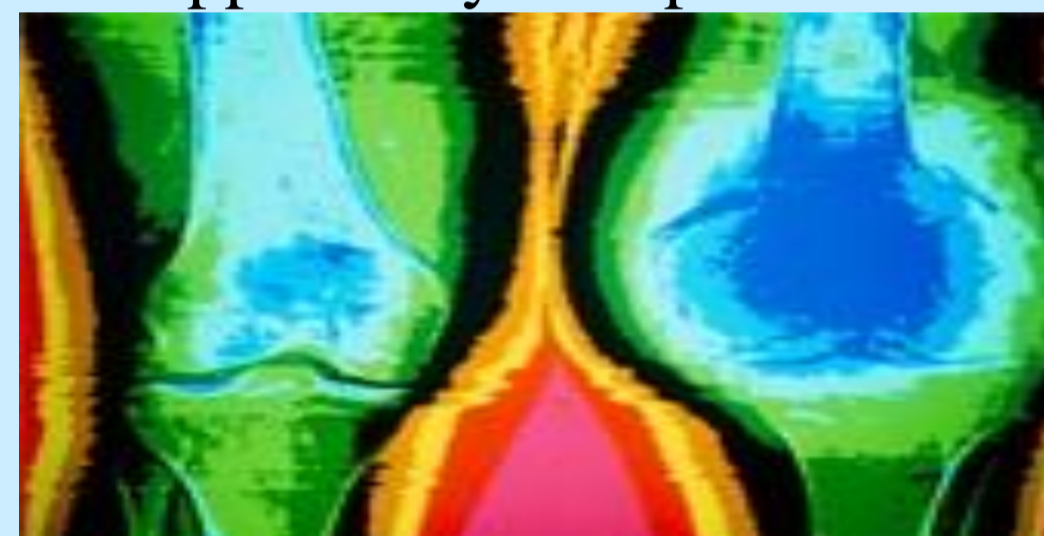
Figure 1: Representation of osteosarcoma in a left knee (blue at center right) on a coloured computed tomography (CT) scan.

Aim

- To explore the availability and clinical usefulness of serum biomarker detection in patients with osteosarcoma in a systematic process.

Systematic literature review → Retrospective clinical study → Pilot laboratory study

Systematic Literature Review

Methods	Results
• Medline and Embase used as search databases. • The search terms were 'osteosarcoma', 'tumour markers', and 'serum' & was limited to human studies in English since 10 years ago. • Animal studies were excluded.	• 22 studies of biomarkers relating to prognosis and clinical outcomes in osteosarcoma were found. • Notable biomarkers include **C - reactive protein (CRP) & alkaline phosphatase (ALP)** to be tested with others in the clinical study.

Retrospective Clinical Study

Methods

134 osteosarcoma patients from the hospital database were retrospectively reviewed. 17 patients were excluded due to incomplete data.

Levels of C-RP, ALP & biomarkers such as albumin & ESR (erythrocyte sedimentation rate) were collated from the first blood test of each patient.

The data was analysed corresponding with the patients' overall survival by plotting survival curves for each biomarker.
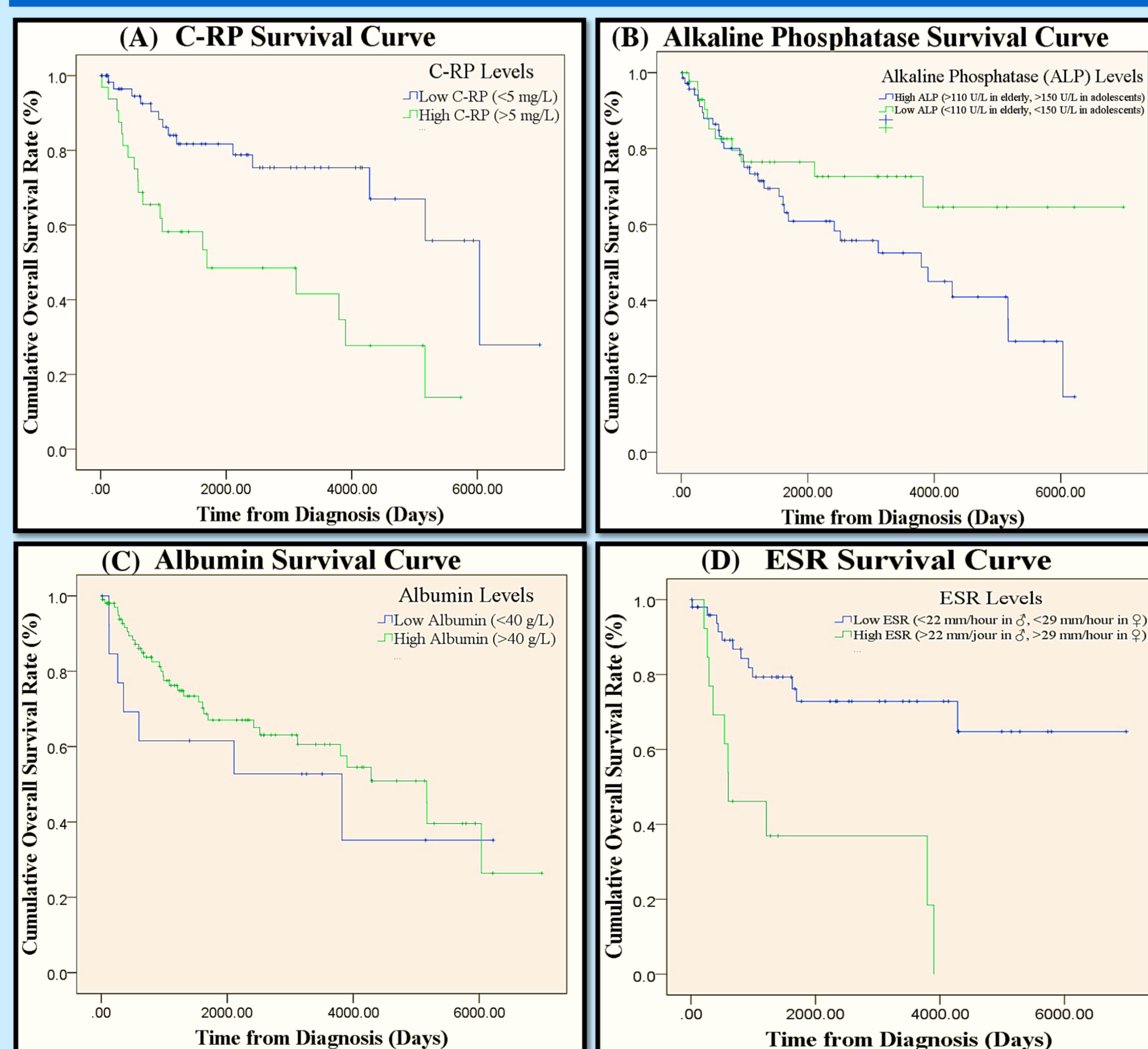
Results/Discussion

(A) C-RP Survival Curve — C-RP Levels: Low C-RP (<5 mg/L), High C-RP (>5 mg/L). Cumulative Overall Survival Rate (%) vs Time from Diagnosis (Days)

(B) Alkaline Phosphatase Survival Curve — Alkaline Phosphatase (ALP) Levels: High ALP (>110 U/L in elderly, >150 U/L in adolescents), Low ALP (<110 U/L in elderly, <150 U/L in adolescents)

(C) Albumin Survival Curve — Albumin Levels: Low Albumin (<40 g/L), High Albumin (>40 g/L)

(D) ESR Survival Curve — ESR Levels: Low ESR (<22 mm/hour in ♂, <29 mm/hour in ♀), High ESR (>22 mm/hour in ♂, >29 mm/hour in ♀)

Figure 2(A-D): **Low levels of albumin** & **high levels of C-RP, ALP & ESR in the blood** were found to correlate with **lower overall survival rates** among **osteosarcoma** patients.

Statistical analysis of T-tests ($P<0.05$) had found significant differences between biomarker levels and survival outcome in C-RP, ALP & ESR but not in albumin.
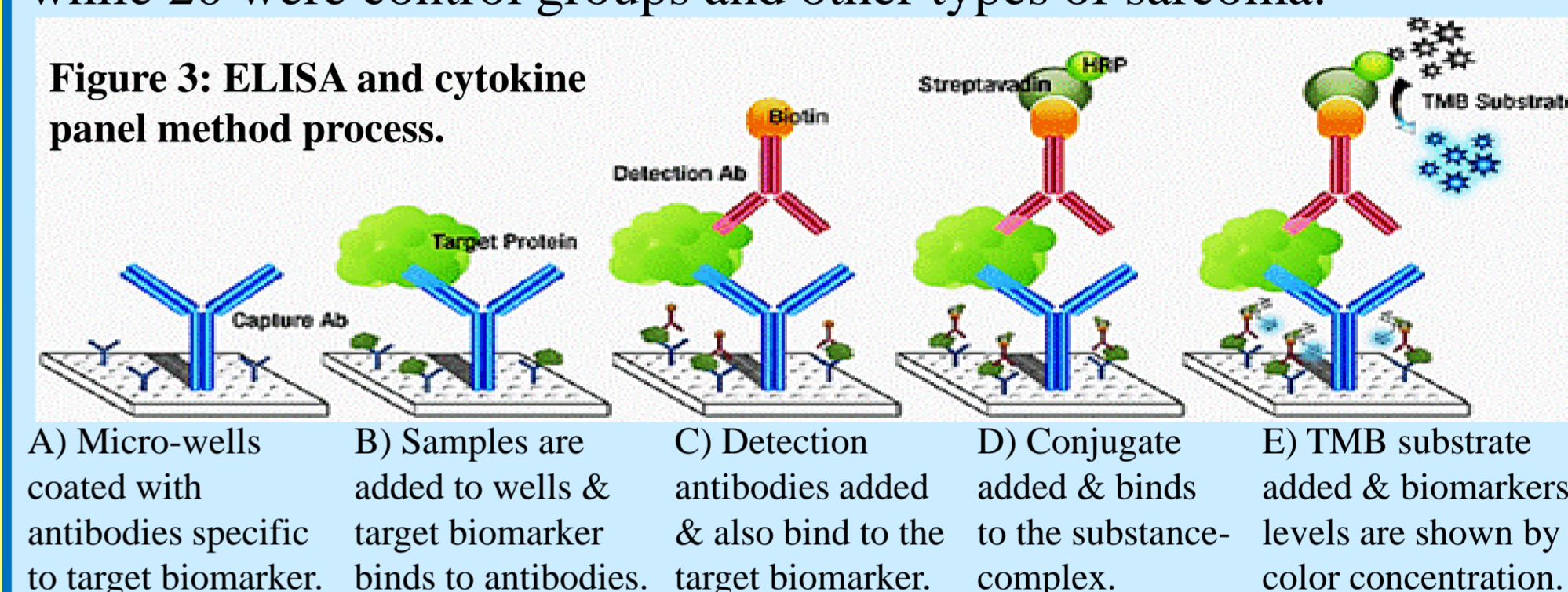
Pilot Laboratory Study

Methods

1) Further interest to explore wider range of biomarkers led to a pilot study to detect levels of chondroitin sulphate proteoglycan (CSPG4) & 10 cytokines using enzyme-linked immunosorbent assays (ELISA) and pro-inflammatory cytokine panel explained below.
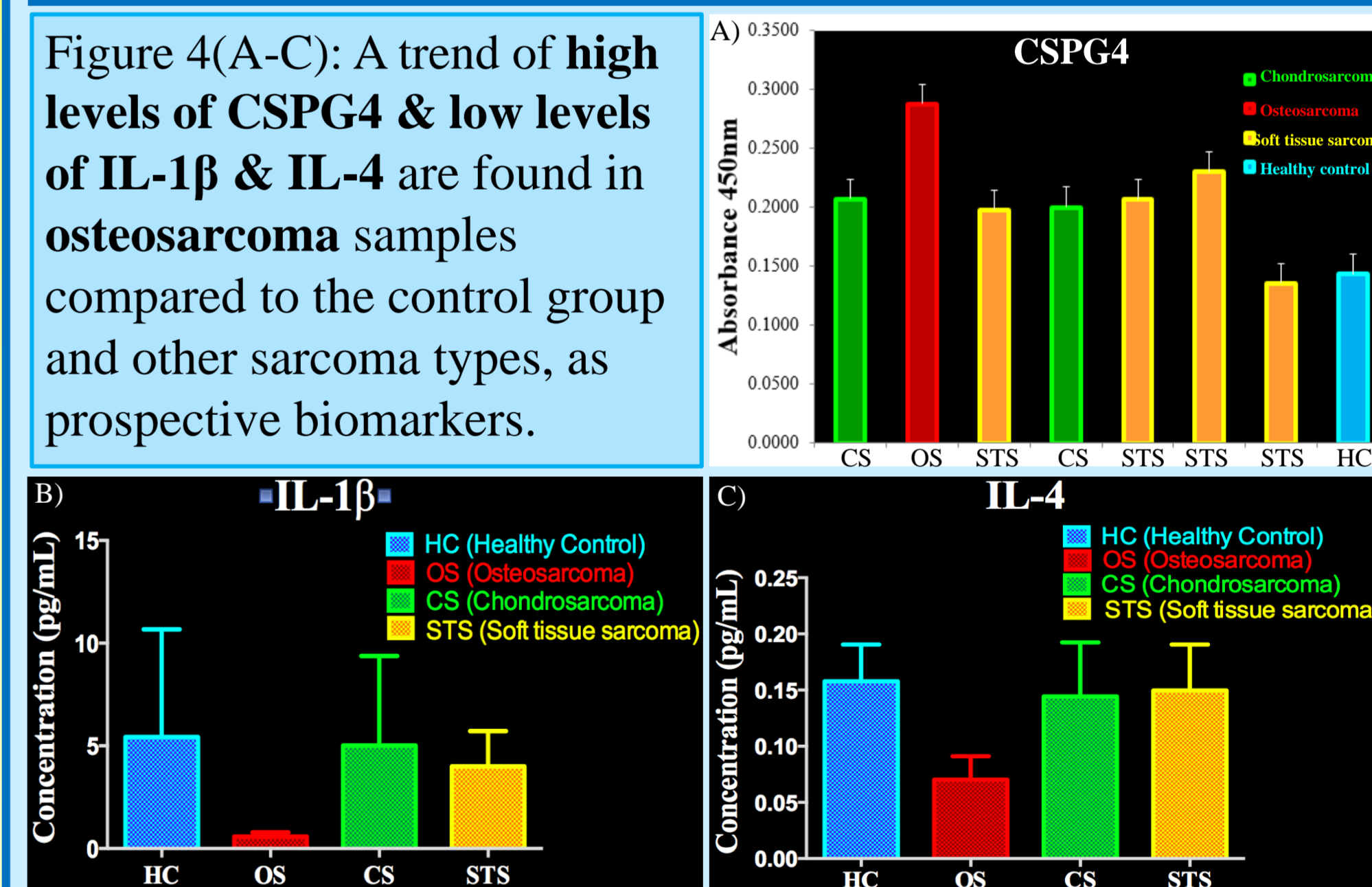
2) 25 blood samples were tested, of which 5 were osteosarcoma while 20 were control groups and other types of sarcoma.

Figure 3: ELISA and cytokine panel method process.

A) Micro-wells coated with antibodies specific to target biomarker.
B) Samples are added to wells & target biomarker binds to antibodies.
C) Detection antibodies added & also bind to the target biomarker.
D) Conjugate added & binds to the substance-complex.
E) TMB substrate added & biomarkers levels are shown by color concentration.

Results/Discussion

Figure 4(A-C): A trend of **high levels of CSPG4 & low levels of IL-1β & IL-4** are found in **osteosarcoma** samples compared to the control group and other sarcoma types, as prospective biomarkers.

A) CSPG4 — Absorbance 450nm. Chondrosarcoma, Osteosarcoma, Soft tissue sarcoma, Healthy control. (CS, OS, STS, CS, STS, STS, STS, HC)

B) IL-1β — Concentration (pg/mL). HC (Healthy Control), OS (Osteosarcoma), CS (Chondrosarcoma), STS (Soft tissue sarcoma)

C) IL-4 — Concentration (pg/mL). HC (Healthy Control), OS (Osteosarcoma), CS (Chondrosarcoma), STS (Soft tissue sarcoma)

Conclusion

The project demonstrated the application of serum biomarkers from current literature & identified new prospective biomarkers of osteosarcoma. This will support future studies in biomarkers to predict patient prognosis and outcomes in osteosarcoma.

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References
1. Bielack S. et al. (2009) 'Osteosarcoma: ESMO Clinical Recommendations for diagnosis, treatment and follow-up', *Annals of Oncology*, 20(suppl 4), 137-139.
2. Kong C. et al. (2009) 'Biomarkers in Osteosarcoma', *Expert Opinion on Medical Diagnostics*, 3(1), 13–23.