Cardiovascular risk and systemic inflammation in male professional rugby: a cross-sectional study

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ABSTRACT

Objective To investigate cardiovascular risk factors’ prevalence and association with systemic inflammation in professional male rugby players (RP).

Methods A cross-sectional investigation of 46 professional male RP (26.1±4.1 years) cardiovascular risk factors were compared by position. Inflammatory markers were compared with healthy controls (n=13) and patients with rheumatoid arthritis (RA) (n=10).

Results Twenty-six per cent of RP had no risk factors, 49% had 1–2 cardiovascular risk factors and 25% had 3–4 risk factors. Forwards had greater body fat (p<0.001), visceral fat (p<0.001), glucose (p=0.025), and C reactive protein (CRP) (p=0.023) compared with backs. RP demonstrated more favourable lipid and glucose profiles than reference values for the general population. Most RP (n=28, 61%) had elevated blood pressure (≥140/90 mm Hg). RP had higher vascular adhesion molecule-1 (VCAM-1) (p=0.004) and intracellular adhesion molecule-1 (ICAM-1) (p=0.002) than healthy controls. RP had lower CRP than patients with RA (p=0.009), while one-third (n=15) displayed equivalent ICAM-1 and VCAM-1 levels. Multivariate clustering and principal component analysis biplots revealed higher triglycerides, inflammatory markers, and worse body composition were associated with forwards.

Conclusions Despite athletic status, most of this rugby cohort had at least one cardiovascular risk factor. Concomitantly, these RP demonstrated increased levels of inflammation, with one-third, primarily forwards, displaying equivalent levels to patients with inflammatory disease. Further studies are needed to unravel the prognostic implications of increased inflammation in RP because unchecked, chronic inflammation may lead to increased cardiovascular disease risk.

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide, prompting public health efforts to promote strategies to reduce incidence and progression. The cardiovascular benefits of regular exercise are well established and primarily associated with moderate exercise (at least 150 mins/week of moderate intensity or 75 mins/week of vigorous intensity aerobic activities). The cardiovascular health of athletes who exceed recommended levels of exercise is not completely understood, particularly in sports such as rugby, where athletes...
are selected for their high body mass and are exposed to repetitive blunt trauma.

Research on the impact of exercise participation that greatly exceeds recommendations on long-term cardiovascular health is limited. Individuals engaged in exercise have a lower all-cause mortality risk than inactive populations. However, a greater risk reduction has been shown for individuals exercising 3–5 times compared with those exercising at ≥10 times. Some studies present a favourable view of longevity in elite athletes, while others suggest a higher prevalence of important cardiovascular risk factors. Participation in sports, such as rugby, involves more than exposure to exercise, and the concomitant impact of repetitive blunt trauma and obligated weight gain remains uncertain and sport specific. Prior work in rugby, American football, and Olympic athletes has demonstrated a high prevalence of elevated blood pressure (BP), visceral fat and dyslipidaemia, which may relate to inflammation, a known cardiovascular risk factor.

Therefore, the primary aim of this study was to characterise cardiovascular risk factors, including novel markers of inflammation, in professional rugby players (RP) according to position due to its known influence on body composition. Forwards are taller and heavier and engage in a higher proportion of isometric tasks, such as rucking and scrummaging. Backs primarily engage in tasks with more of an isotonic component, such as high-intensity running. Our secondary aim was to compare inflammatory makers in RP to healthy controls (HC) and patients with rheumatoid arthritis (RA) to provide context without comparable research in athlete populations. We hypothesised that RP, particularly those playing forward position (with associated higher body mass and visceral fat), would exhibit greater cardiovascular risk and some degree of elevation in inflammatory markers as compared with controls but that inflammatory markers would not be as high as in patients with a known inflammatory disease.

METHODS

Study design

This cross-sectional study included RP from one top-tier rugby team. Two comparator groups were used, including HC and patients with established RA. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Patients or the public were not involved in our research’s design, conduct, reporting or dissemination plans.

Participants

The sample included active, professional male rugby union players. RP were categorised based on primary position: forward or back. The inclusion criteria included RP aged ≥18 years and signed to a current professional rugby contract. Participants were excluded if they had a current acute injury within a 6-week postoperative period at the time of testing or if they registered a fever or illness on the day of testing. Markers of inflammation were compared with HC and patients with established RA from a biobank dataset at Trinity Biomedical Sciences Institute. All HC and patients with RA with inflammatory biomarker data available from retrospective blood sampling and storage to the biobank were included. HC and patients with RA were not matched for age or sex due to unavailable data within the biobank. Blood samples for this biobank were collected between November 2020 and April 2021 from patients recruited from the Rheumatology Department, St. Vincent’s University Hospital, and HC recruited at Trinity Biomedical Sciences Institute and St. Vincent’s University Hospital. Blood samples were collected in the early morning following a 12-hour fast. All participants gave fully informed written consent. The research was performed in accordance with the Declaration of Helsinki.

Data collection

Data were collected from RP in the early morning (07:00 to 09:00) during pre-season (2020) at the club’s medical facilities. Demographic information obtained included age, self-reported ethnicity, height (cm), weight (kg) and body mass index (BMI) (weight (kg)/height (m)²).

Dual-energy X-ray absorptiometry (DXA) scans (Lunar iDXA, GE Healthcare, Madison, Wisconsin, USA) of RP were taken at the end of the pre-season (December 2020). Standard scanning protocols and procedures were used to ensure maximum reliability. One skilled technologist conducted all scans following the manufacturer’s guidelines. Scans were checked by a second skilled densitometrist, certified in clinical densitometry. Analyses were conducted using GE Lunar EnCore software (V.15.0) for total (kg), lean (g) and fat (g) masses and advanced CoreScan software (EnCore V.15.0, GE Lunar Healthcare, Madison, Wisconsin) for estimated visceral fat (g).

BP was measured before DXA scans using an electronic automated monitor (Omron 705IT, Omron Corporation, Kyoto, Japan) following European Society of Cardiology (ESC) guidelines. Reported values represent the average of triplicate measurements.

During pre-season screening, venous blood samples and fingerstick blood samples were collected following a 12-hour fast by a Sports and Exercise Medicine Specialist Registrar. RP were allowed to consume water during their fast and were void of exercise for at least 24 hours. Body temperatures were taken due to COVID-19 protocols. RP were screened for current injury status. Samples were collected in two 2.5 mL VACUETTE tubes: one red Z serum clot activator coated with silica particles (Greiner Bio-One 455092) and one green lithium heparin separator (Greiner Bio-One 455092). Samples were aliquoted and stored at −80°C after preparation and collection. Routine ELISA were performed for troponin I, interleukin 6 (IL-6) and neuropeptide Y. MSD multiplex ELISA (Meso Scale Diagnostics) were performed for C reactive protein (CRP), intercellular adhesion
molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and serum amyloid A (A-SAA). Lipid and glucose were assessed using CardioChek PA analyser (Polymer Technology Systems, Indiana, USA; BHR Pharmaceuticals, Nuneaton, UK), a reliable tool for CVD screening. Measurements were taken from a fingerstick blood sample, and analysis was performed according to the manufacturer’s instructions. Lipid and glucose measures were classified following ESC guidelines.

RISK FACTOR DEFINITION

The CVD risk factors were defined according to ESC guidelines, and the QRSK-3 lifetime risk tool (https://qrisk.org/lifetime/index.php) was used to establish lifetime CVD risk.

- **Overweight/obesity:** Body fat percentage (%BF) ≥20%, measured by DXA and BMI ≥25 kg/m².
- **Hypertension:** Systolic BP (SBP) ≥140 mm Hg and/or diastolic BP (DBP) ≥90 mm Hg or subjects that were in treatment with antihypertensive drugs.
- **Dyslipidaemia:** Low-density lipoprotein (LDL) ≥130 mg/dL and/or high-density lipoprotein (HDL) ≤40 mg/dL.
- **Hypoglycaemia:** Fasting glucose ≥100 mg/dL or current treatment with insulin or antidiabetic drugs.
- **Smoking:** Current or former tobacco smoker.
- **Age:** No risk, young (18–29 years) and risk, increased age (30–45 years), arbitrarily defined.
- **Elevated CRP:** Lower risk, 1–3 mg/L; higher risk, >5 mg/L; and acute inflammation: >10 mg/L.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software (V.22; SPSS) and R Statistical Software (Foundation for Statistical Computing, Austria). Before analyses, assumptions of normality were made using the Shapiro-Wilk test (p>0.05) and visualisations through histograms and Q–Q plots. Parametric testing was used for normally distributed variables, and non-parametric testing for variables not normally distributed (ie, CRP, IL-6, ICAM-1, VCAM-1 and A-SAA). Continuous data are expressed as mean±SD or median and IQR. Categorical data are expressed as number of observations and frequencies (n, %).

Descriptive statistics were calculated to evaluate anthropometric characteristics and the prevalence of risk factors. RP were categorised based on position, forward or back, to evaluate the effects of position. Differences in risk factors between positions were evaluated using independent samples t-test or Mann-Whitney U test. Differences between proportions were calculated by a χ² test. One-way analysis of variance (ANOVA), followed by a Tukey’s multiple comparison test, was used to analyse serum concentrations of VCAM-1 and CRP, HC and patients with RA. A Brown-Forsythe ANOVA, followed by a Dunnett’s multiple comparison, was used to analyse serum concentrations of CRP, A-SAA and ICAM-1 for RP, HC and patients with RA.

Additionally, players were categorised based on the distribution of risk factors identified above: no risk factors, one to two risk factors, and three to four risk factors. Bioinformatic analysis, including hierarchical clustering and biplot analysis, was performed in R utilising packages gplots (v3.1.1) and ggbiplot (v0.55), respectively. Euclidian measures with complete agglomeration were used for hierarchical clustering (function: heatmap.2) following data scaling. Biplots combine principal component analysis (PCA) plots and a loading plot. Significance for all analyses was defined as p≤0.05.

RESULTS

Table 1 provides details of demographic and clinical characteristics for RP (n=46) by position, forwards (n=21) and backs (n=25). No player received treatment for a current musculoskeletal injury within 6 weeks postoperative period or registered a fever.

Forwards had greater values for all measures of body composition, including %BF (p<0.001) and visceral fat (p<0.001) than backs. Using reference intervals for male RP matched for age, visceral fat fell on the 50th percentile for backs (399.6±180.4 g) and between the 50th and 97.5th percentile for forwards (657.9±399.6 g). Forwards had significantly more RP categorised as overweight/obese (5 vs 0, p=0.001).

SBP (forwards: 132.9±8.6 vs backs: 132.2±11.2 mm Hg, p=0.894) and DBP (forwards: 75.5±8.2 vs backs: 75.8±12.3 mm Hg, p=0.977) did not differ between playing positions. Most RP (n=28, 61%) had elevated BP (≥140/90 mm Hg) with no difference in distribution between positions (forwards: 33% vs backs: 28%, p=0.841).

There was no difference in lipid profiles between positions. No player had total cholesterol (>200 mg/dL) or triglyceride (≥150 mg/dL) values above optimal levels. Four RP, all forwards, had LDL above the optimal range (>100 mg/dL).

Compared with reference goal values suggested by the ESC, RP had more favourable values for total cholesterol by −35% (129.3 vs <200 mg/dL), LDL by −30% (70.2 vs <100 mg/dL), triglycerides by −53% (70.9 vs <150 mg/dL), and HDL by +25% (49.8 vs >40 mg/dL). Eight RP (17.4%) met the criteria for dyslipidaemia due to low HDL as opposed to high LDL with no difference in distribution between playing positions (forwards: 19% vs backs: 16%, p=0.809). Forwards had higher glucose values compared with backs (68.9±16.1 vs 56.6±17.9 mg/dL, p=0.025) (Table 1) with one player (forward) with glucose above optimal values (>100 mg/dL).

Forwards had higher CRP compared with backs (p=0.023). No other inflammatory marker was significantly different between positions. Thirty RP (65%) had CRP >3 mg/L, with forwards being more likely to have CRP >3 mg/L than backs (95% vs 60%, p=0.051). Ten RP (21.7%), five forwards and five backs had CRP values suggestive of acute inflammation (>10 mg/L), with three of these RP recovering from surgery (3–6
Five RP (10.9%), four backs and one forward had IL-6 values above normal (range: 22.5–137.9 pg/mL) (table 1). Most RP (74%) had ≥1 risk factors, with 25% identified with 3–4 risk factors. The most common risk factor was elevated CRP, hypertension, increased age, dyslipidaemia and elevated %BF (table 2). Using the QRISK-3 lifetime risk tool, the average risk percentage was 30.2% (wards=33.2%, backs=27.3%), ranging from 40% (forward) to 23.6% (back).

RPs’ CRP, ICAM-1, VCAM-1 and A-SAA were compared with HC (n=13) and RA (n=10). RP had significantly higher values for VCAM-1 (p=0.004) and ICAM-1 (p=0.002) compared with HC (figure 1 and table 3). Compared with patients with RA (n=10), RP had significantly lower CRP (p=0.009) compared with RA (figure 1 and table 3). A non-significant trend of increased VCAM-1 in RP compared with RA groups was observed. One-third of RP (n=15) had ICAM-1 and VCAM-1 concentrations similar to patients with RA (figure 1). Half of RP, predominately forwards, had CRP concentrations exceeding the upper limit of normal for individuals with known inflammatory diseases (5 mg/L), such as RA.25
Multivariate clustering analysis was used to visualise the pattern of cardiovascular risk factors generated through classification (playing position and number of risk factors), and clustering was applied to group RP (43 RPs with data for all cardiovascular variables) according to cardiovascular data. RP categorised as forwards, and those with 3–4 risk factors display higher values for triglycerides, inflammatory markers and body composition. In contrast, most backs displayed lower values for risk factors and a more even distribution of risk factors. Four main clusters were identified, including cluster 1, triglycerides, ICAM-1 and VCAM-1; cluster 2, CRP and A-SAA; cluster 3, glucose, visceral fat, BMI and %BF; and cluster 4, total cholesterol, LDL, HDL, SBP and DBP (figure 2).

Biplots, a combination of a PCA plot and a loading plot, were used to assess the potential overall correlation of specific risk factors and position and the potential

| Table 2 | Prevalence of risk factors by position |
|-----------------|-----------------|-----------------|
|                | Forwards (n=21) | Backs (n=25) | P value |
| Overweight/obesity, n (%) | 5 (24%) | – | <0.001* |
| Hypertension, n (%) | 7 (33%) | 7 (28%) | 0.841 |
| Dyslipidaemia, n (%) | 4 (19%) | 4 (16%) | 0.869 |
| Hyperglycaemia, n (%) | 1 (5%) | – | 0.253 |
| Smoking, n (%) | – | – | – |
| Increased age, n (%) | 5 (24%) | 4 (16%) | 0.422 |
| Elevated CRP, n (%) | 18 (86%) | 12 (48%) | 0.035* |

Data are presented as n (%), and χ² test (proportions) was used for statistical analysis. *P ≤ 0.05 forwards versus backs.

CRP, C reactive protein.

Figure 1 Serum levels of proinflammatory markers in rugby players (RP) compared with patients with rheumatoid arthritis (RA) and healthy controls (HC). Serum concentrations of CRP, A-SAA, VCAM-1 and ICAM-1 of rugby players (RP), rheumatoid arthritis patients (RA) and healthy controls (HC) are shown. A one-way ANOVA (VCAM-1) and a Brown-Forsythe ANOVA (CRP, A-SAA and ICAM-1) was used for statistical analysis, p values <0.05 were considered significant, points indicate individual samples, n= 10-46/group. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.
relationship between risk factors. Biplots of the first two principal components demonstrated overlapping clusters of forwards and backs. For the biplot assessing established risk factors (figure 3A), the first and second PCA axes account for 25.8% and 18.4% of the total variance, respectively. LDL, total cholesterol, %BF, BMI and visceral fat are the most influencing risk factors for the first PCA. For the biplot assessing inflammatory markers and visceral fat (figure 3B), the first and second PCA axes explain 44.1% and 27.7% of the total variance, respectively. The forward position had an increased association with CRP, A-SAA, ICAM-1 and VCAM-1, suggesting a relationship with the level of inflammation.

**Table 3** Markers of inflammation in healthy controls and rugby players by position

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>RP (n=46)</th>
<th>HC (n=13)</th>
<th>RA (n=10)</th>
<th>Result</th>
<th>RA × RA P value</th>
<th>RA × HC P value</th>
<th>RP × HC P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, pg/mL</td>
<td>17 856 ± 17 229</td>
<td>1 245 733 ± 1951 441</td>
<td>68 360 000 ± 53 076 301</td>
<td>F (2.000, 9.023) = 15.73</td>
<td>0.009</td>
<td>0.722</td>
<td>0.009</td>
</tr>
<tr>
<td>ICAM-1, pg/mL</td>
<td>775 150 ± 496 864</td>
<td>495 440 ± 156 978</td>
<td>1 299 566 ± 669 681</td>
<td>F (2.000, 13.07) = 8.04</td>
<td>0.102</td>
<td>0.002</td>
<td>0.011</td>
</tr>
<tr>
<td>A-SAA, pg/mL</td>
<td>5 514 758 ± 7 160 014</td>
<td>3 573 340 ± 2 982 059</td>
<td>1.07E+09 ± 1.77E+09</td>
<td>F (2.000, 9.000) = 3.65</td>
<td>0.227</td>
<td>0.340</td>
<td>0.225</td>
</tr>
<tr>
<td>VCAM-1, pg/mL</td>
<td>961 105 ± 530 928</td>
<td>570 288 ± 159 246</td>
<td>777 978 ± 273 085</td>
<td>F (2, 66) = 5.90</td>
<td>0.353</td>
<td>0.004</td>
<td>0.371</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. One-way analysis of variance (ANOVA), followed by a Tukey’s multiple comparison test, was used for analysis of serum concentrations of VCAM-1 and a Brown-Forsythe ANOVA, followed by a Dunnett’s multiple comparison, was used for analysis of serum concentrations of CRP, A-SAA and ICAM-1. A-SAA, serum amyloid A; CRP, C reactive protein; ICAM-1, intercellular adhesion molecule 1; RA, rheumatoid arthritis; RP, rugby players; VCAM-1, vascular cell adhesion molecule 1.

**DISCUSSION**

In this study, we aimed to assess the prevalence of cardiovascular risk factors and their association with systemic inflammation in professional RP, according to playing position. Despite their status as an athlete, most of this cohort had at least one cardiovascular risk factor. The most prevalent risk factor was elevated CRP, followed by hypertension, increased age, dyslipidaemia, categorised by low HDL, and overweight, categorised by increased BF%. Despite the absence of acute injury or diagnosis of an inflammatory disorder, RP, primarily forwards, had increased levels of systemic inflammation, with one-third displaying equivalent levels of inflammation to patients with an established, chronic inflammatory disease. RP with increased body mass (forwards) were more likely to have more cardiovascular risk factors and increased inflammation, supporting our hypothesis.

Although young athletes are often regarded as the model of cardiovascular health, recent research has challenged this viewpoint. An accumulation of observational data has reported hypertension and cardiometabolic syndrome in athletes across sporting disciplines. Specifically, Ascenzi et al (2019) reported an unexpected cardiovascular risk in Olympic athletes from power, skill and mixed-discipline sports. Hypertension during young adulthood, the period coinciding with professional rugby participation, is a well-established independent risk factor for later-life CVD morbidity and mortality. In this study, more than half of RP had elevated BP, with one-third classifying as hypertensive. Previous research has described the potential development of a pathological cardiovascular phenotype in professional American football players due to weight gain and increased SBP, resulting in concentric left ventricular hypertrophy, arterial stiffening and reduced left ventricular diastolic function. Although long-term implications remain unknown, further investigation is warranted due to similarities between rugby and American football. Several studies stressed the potential role of inflammation associated with prolonged exposure to high-intensity exercise on cardiovascular health. To our knowledge, this study is not only the first to investigate the cardiovascular health of RP but the first to include a comprehensive investigation of inflammation alongside traditional CVD risk factors.

Higher levels of inflammation were identified in forwards, with significantly higher CRP values. In the absence of comparable research on RP, we compared inflammatory markers to a small cohort of HC and patients with RA to contextualise findings. Soluble VCAM-1 and ICAM-1 were significantly higher in RP, primarily forwards, compared with HC, with one-third (n=15) of RP displaying similar if not higher levels compared with patients with RA, of which three were on disease-modifying antirheumatic drugs and the remaining were treatment naïve. Attendant differences in body composition due to position-specific demands provide a potential latent rationale for higher levels of inflammation in forwards. VCAM-1 and ICAM-1 are cellular adhesion molecules that mediate circulating leukocytes’ adhesion and transendothelial cell migration. Higher levels are known to be increased at sites of inflammation, with
several studies showing an association between increased circulatory soluble ICAM and VCAM and cardiovascular risk.\textsuperscript{30} An increase in both adhesion molecules has been observed in patients with RA, levels of which decrease following therapeutic intervention.\textsuperscript{31} Increased levels have been shown to predict future cardiovascular events in patients with RA.\textsuperscript{32} Thus, the observed increase in circulating levels in RP suggests increased levels of inflammation. Although inflammation is a normal response to engagement in high-intensity exercise or the occurrence of an injury, both common in rugby, exposure to chronic systemic inflammation is an early marker of endothelial dysfunction contributing to atherosclerosis progression.\textsuperscript{7} How inflammation affects endothelial cell activity in athletes remains unknown, although intensive training is proposed to increase platelet aggregation excessively. Our findings provide the foundation for further investigation into the role of prolonged low-grade systemic inflammation on the long-term cardiovascular health of elite athletes due to potential future clinical concerns.

We identified that RP mean visceral fat values fell between the 50th and 97.5th percentile, where being closer to the first percentile is preferred for cardiometabolic health,\textsuperscript{24} with forwards having significantly higher values than backs. Increased cardiovascular risk with increasing body mass is well established in the general population and, more recently, in retired athletes.\textsuperscript{10} Further, visceral adiposity has been found to influence
the inflammatory process by increasing adipocytokine production and proinflammatory activity \(^{35}\) and is thus associated with the development of low-grade systemic inflammation \(^{94}\) and increased cardiovascular risk. \(^{35}\) In this study, forwards were identified with a greater number classifying as overweight/obese (defined as \(\%BF \geq 20\% \) and \(\text{BMI} \geq 25 \text{kg.m}^{-2}\)), higher visceral fat and elevated CRP compared with backs. This suggests that RP with increased size, mass and visceral fat associated with position-specific demands may be more susceptible to increased cardiovascular risk.

**Limitations**

There are several limitations to this study. Due to the exploratory nature of this study, RPs from only one top-tier rugby team were included, resulting in a small sample size, limiting the ability to generalise findings. No control group was used to compare cardiovascular risk factors. Markers of inflammation were compared with data from HC and patients with RA extracted from a biobank database to provide context to findings in the absence of comparable research in athletes. This biobank database did not include demographic profiles; therefore, these were not matched for age or sex. Findings are compared with established risk factor criteria from the general population due to no established criteria for athletic populations. Although efforts were made to account for confounding factors that may contribute to increased inflammation, including injury status and the presence of fever, inflammatory markers were assessed at one-time points, therefore representing a snapshot of inflammatory status. Additionally, 3 of the 10 RP with acute inflammation (CRP>10 mg/L) were 3–6 months postoperative; therefore, cautious interpretation is warranted. Finally, although the club’s nutritionist monitors RP’ diets, it was not possible to investigate the impact of salt intake, supplement intake and use of nonsteroidal anti-inflammatory drugs.

**CONCLUSION**

Despite their status as professional RP, many RP had cardiovascular risk factors. Among the prevalent risk factors were hypertension and dyslipidaemia, and healthcare providers should consider assessment for these when performing preparticipation screening and target modifiable risk factors. One-third of RP’s, primarily forwards, displayed equivalent levels of inflammation to patients with established inflammatory disease. Future studies should aim to unravel the mechanistic drivers of inflammation in professional athletes and prognostic implications due to the potential for increased CVD risk.

By assessing the limits, a more complete picture of the processes underpinning the health benefits of exercise can be revealed, which can inform translational research and benefit both athletes and the general population.

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