LETTER TO THE EDITOR

IS THERE AN ASSOCIATION BETWEEN BIOCHEMICAL PARAMETERS AND PROSTATE-SPECIFIC ANTIGEN "GREY ZONE"? AN ITALIAN PILOT STUDY

S. TARTAGLIONE[1*], E. BERARDELLI[1*], C. DE VITO[2], A. NARDI[2], G. GENNARINI[1], G. GIRELLI[3], A. ANGELONI[4] and E. ANASTASI[1]

[1]Department of Molecular Medicine, Policlinico Umberto I, "Sapienza" University, Rome, Italy; [2]Department of Public Health and Infectious Diseases, "Sapienza" University, Rome, Italy; [3]Immunohematology and Transfusion Medicine Unit, Policlinico Umberto 1, "Sapienza" University, Rome, Italy; [4]Department of Experimental Medicine, Policlinico Umberto I, "Sapienza" University, Rome, Italy

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[*]These Authors contributed equally to this paper

To the Editor,

Prostate-specific antigen (PSA) is a type of serine protease produced by prostate epithelial cells. It represents the most frequently used tumor marker in early detection of prostate cancer (PC), the fifth leading cause of cancer death among men worldwide. This biomarker is useful in the identification of recurrent disease after local therapy and in the management of advanced disease as well as in the evaluation of the future risk of PC development (1). PSA is characterized by high sensitivity but unfortunately it has low specificity: in fact, it can increase in PC but also in cases of inflammation or prostate infections. Commonly, 4 ng/mL is used as a threshold value for PSA even if it has been reported that 20% of patients with low grade PC have PSA values < 4 ng/mL and only 25% of patients affected by PC show PSA levels of 4–10 ng /mL: this range is defined as a "grey zone" for PC risk (2). The clinical management of men having PSA levels in the "grey zone" is challenging: this population often undergoes invasive and expensive diagnostic investigations such as digital rectal examination, transrectal ultrasonography and particularly prostate biopsy, which has frequent and multiple complications. PSA levels may be influenced by age and BMI but mostly by several clinical conditions, i.e. prostate inflammation and infections or benign prostate hyperplasia (BPH), which deserve careful attention in the interpretation of test results (1). Moreover, several studies recently investigated the relationship between PSA and biochemical parameters. For example, in a few scientific studies it was found that higher serum ferritin levels were positively associated with increased serum PSA levels and risk of PC (3). Some papers described a proportional increase of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides in PC patients with high PSA levels, although the exact roles of different lipid fractions on PC aggressiveness should be further evaluated (4). Lately, many studies focused their attention on serum 25(OH)Vitamin D and PC and the results are still controversial (5). 25(OH)Vitamin

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Corresponding Author:
Dr Emanuela Anastasi,
Department of Molecular Medicine,
"Sapienza" University, Viale Regina Elena 324,
00161 Rome, Italy
Tel.: +39 064472347 - Fax: +39 064478381
e-mail emanuela.anastasi@uniroma1.it

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D deficiency has been associated with augmented risk of PC for men with elevated serum PSA levels, but other findings conversely suggested that men with higher 25(OH)Vitamin D serum levels were at increased risk of developing PC (6). It is known that PC risk is higher in men with PSA of 4 to 10 ng/mL but in clinical practice it could be difficult to distinguish PC from BPH based on PSA levels in this range (2). As a consequence, men having PSA "grey zone" often undergo many unnecessary medical procedures, and it would be useful to better characterize this population of subjects to possibly identify which patients really need a prostate biopsy, and biochemical parameters could represent a non-invasive and economic tool (1). The aim of this study is to evaluate a potential association between PSA and certain biochemical parameters, comparing serum values of each analyte in two groups of healthy Italian men: one with PSA normal range *vs* one other with PSA "grey zone".

MATERIALS AND METHODS

Subjects

This research was designed as a cross-sectional study. Between 2015 and 2018, 1421 blood donors aged between 40 and 70 (mean±SD, 54.71±5.63) were enrolled at the transfusion center of Policlinico Umberto I, Sapienza University of Rome (latitude 41°54′39″24 N). Informed consent was obtained from all participants in the study. Subjects selected for this research were healthy males: we chose blood donor volunteers since they are by definition presumably normal subjects, since before donation they are checked also for possible biochemical abnormalities. At enrollment the medical history of the subjects was collected through their statements, and weight, height and blood pressure were recorded. Specific inclusion criteria for our selected population were: having no present or past PC or malignant diseases, no symptoms suggestive of prostatic disorders, no drugs that affect mineral or iron metabolism, no use of any lipid lowering medications or 25(OH)Vitamin D supplementation. We selected only subjects making one donation per year since it is known that ferritin values are affected by this feature. Peripheral blood samples were drawn and immediately sent to the laboratory of Tumor Markers of the Policlinico Umberto I, Sapienza University of Rome and all men were screened for PSA. Among 1,421 blood donors tested for PSA two subgroups were identified: 63 subjects with PSA between 4 and 10 ng/mL and 69 subjects with PSA levels lower than 4 ng/mL. The two groups were homogeneous in terms of age and body mass index. Both populations were tested for lipid profile, ferritin and 25(OH)Vitamin D. The study protocol was approved by our local Institutional Review Board and was performed in accordance with the Declaration of Helsinki.

Methods

Body mass index (BMI) was calculated by the person's weight in kilogram, divided by height in square meters. Peripheral blood samples were collected into 5 mL Red Top Vacutainer tubes with separator gel (Becton, Dickinson and Company, Plymouth, UK), clotted 60 – 90 min and centrifuged for 15 min at 3500 x g. The resulting serum fractions were aliquoted in 1.5 mL Eppendorf tubes (Eppendorf srl, Milano, Italy) and stored at – 80°C until analysis. PSA concentrations were determined using the Access Hybritech PSA kit (Beckman Coulter, Brea, CA, USA), which is World Health Organization (WHO) standardized (PSA WHO 96/670). All tests were run on the automatic chemiluminescent enzyme immunoassay (CLEIA) instrument Access® 2 Immunoassay System (Beckman Coulter, Brea, CA, USA). The detection range was between 0,008–150 ng/mL, with intra-assay coefficient of variation (CV) < 7 % based on the 95% confidence interval, according to manufacturer's specifications.

Ferritin, total cholesterol, HDL, LDL and triglyceride concentrations were measured by electrochemiluminescence immunoassays on the Cobas®E601 chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Calibrators were obtained from Roche Diagnostics. The intra-assay CV was < 5% for each kit utilized. The detection range were 5-1000 µg/L, 3.86-800 mg/dL, 3.09-150 mg/dL, 3.87-549 mg/dL, 8.85-885 mg/dL, respectively, for ferritin, total cholesterol, HDL, LDL, triglycerides. Quantitative determination of serum 25(OH)Vitamin D was performed using the LUMIPULSE G 25(OH)Vitamin D kit (Fujirebio, Tokyo, Japan) on a Lumipulse® G1200 (Fujirebio-Europe, Gent, Belgium) which is an automated assay instrument based on CLEIA technology by a two-step sandwich in

immunoreaction cartridges (7). The detection range was between 4–150 ng/mL, with intra-assay CV < 3% based on the 95% confidence interval, according to manufacturer's specifications. 25(OH)Vitamin D levels were categorized in insufficient (< 20 ng/mL) and sufficient (≥20 ng/mL) according to the latest guidelines published by the Food and Medicine Board at the National Institute of Medicine.

Statistical analysis

Descriptive statistics of the two groups were presented as mean and standard deviation (SD), except for age that was presented as median and interquartile range. Normality was tested using Shapiro-Wilk test. In order to assess differences between the two groups regarding age, BMI and biochemical analytes (total cholesterol, HDL, LDL, triglycerides, ferritin and 25(OH)Vitamin D), Student's *t*-test was applied and differences were tested by the Mann–Whitney U test. If results between the two tests were consistent, only P-value of the Student's *t*-test was reported. Differences for dichotomized 25(OH)vitamin D (in < 20 ng/mL or ≥20 ng/mL) were assessed using *Chi*-squared test. To determine which variables were independently associated with PSA "grey zone" a logistic regression model was built, including all variables with P < 0.25 at the univariate analysis and all those variables considered relevant by a biological point of view, as proposed by Hosmer and Lemeshow. A second logistic regression model considering 25(OH)Vitamin D ≥ 20 ng/mL as the dependent variable was built, including all the preceding variables and seasonality, dichotomized in autumn-winter and spring-summer. Significance level was set at the 0.05. All statistical analyses were performed using the STATA software (version 15.1; STATA Corp., College Station, TX).

RESULTS

PSA values between 4 and 10 ng/mL were found in 4.4% (63) of the 1,421 healthy men enrolled in our study. None of the subjects showed PSA >10 ng/mL. The group of men with PSA "grey zone" had a median age of 58 (IQR 54-61) and a mean BMI of 26.04±2.52. Among the remaining 1,358 studied, blood donors with PSA values <4ng/mL, a group of subjects were selected similar for both age (median 56; IQR 52-60) and BMI (mean 26.05±2.83) to the population of men with PSA "grey zone". Student's *t*-test results, consistent with Wilcoxon signed-rank non-parametric test results, showed no statistically significant difference for age and BMI between men in "grey zone" and the selected population of 69 subjects with PSA <4 ng/mL. None of the biochemical analytes showed statistically significant differences between the two groups for both Student's *t*-test and Mann–Whitney U test non-parametric test. Mean values, standard deviations and the P values of the Student's *t*-test for age, BMI and the biochemical analytes are reported in Table I. Furthermore, although in the PSA "grey zone" group there was higher frequency of subjects with 25(OH) Vitamin D level ≥ 20 ng/mL than in men with PSA < 4 ng/mL (55.56% *vs* 44.44%) this difference was not statistically significant (p= 0.47) (Not shown in table). We further investigated the possible association between age, BMI, biochemical analytes and PSA values. Logistic regression model showed no association between all the selected parameters and PSA in the "grey zone". However, even if it did not achieve statistical significance, 25(OH)Vitamin D ≥20 ng/mL showed the highest association with "grey zone" PSA (OR 1.38, C.I. 0.67 - 2.84) (Table II). We formulated a model testing 25(OH)Vitamin D ≥20 ng/mL as the dependent variable and we found no statistically significant associations except for seasonal variability: 25(OH)Vitamin D values were higher when tested in the spring-summer period than in the autumn and winter months (P = 0.04). The results are shown in Table III.

DISCUSSION

In the present study we did not find any statistically significant difference between "grey zone" population *versus* the "normal range" group regarding the levels of some common biochemical analytes (ferritin, total cholesterol, HDL, LDL, triglycerides and 25(OH)Vitamin D) which, in the scientific literature, have been more frequently associated with modifications of PSA levels. This paper constitutes the first report which tried to better characterize biochemical parameters of this particular population at increased risk of developing PC, the most common malignancy of the male reproductive

Table I. *Comparisons of age, BMI and biochemical analytes between the two groups (PSA < 4 ng/mL and "grey zone" PSA)*

Characteristics	Men with PSA < 4 ng/mL (N = 69)	Men with PSA "grey zone" (N = 63)	P*
Age (years)	56 (52-60)	58 (54-61)	0.075
BMI (kg/m^2)	26.05 ± 2.83	26.04 ± 2.52	0.969
Ferritin (µg/L)	75.23 ± 53.38	63.71 ± 38.05	0.159
Total cholesterol (mg/dL)	201.19 ± 38.17	211.03 ± 34.19	0.122
HDL (mg/dL)	57.06 ± 12.19	59.33 ± 11.99	0.282
LDL (mg/dL)	121.99 ± 33.27	129.43 ± 33.91	0.205
Triglycerides (mg/dL)	106.97 ± 60.46	98.63 ± 47.96	0.384
25(OH)Vitamin D (mg/dL)	20.33 ± 8.74	19.48 ± 6.70	0.531

*Data are reported as mean±standard deviation except for age that is reported as median and interquartile range. BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein. P < 0.05 was considered statistically significant. *Results of the Student's t-test.*

Table II. *Logistic regression analysis assessing associations between examined variables and PSA "grey zone"*

Variables	OR	95% C.I.	P
Age (years)	1.06	0.98 – 1.13	0.130
BMI (kg/m^2)	1.02	0.88 – 1.18	0.780
Ferritin (µg/L)	0.99	0.99 – 1.00	0.272
Total cholesterol (mg/dL)	1.02	0.99 – 1.05	0.243
HDL (mg/dL)	0.99	0.95 – 1.04	0.747
LDL (mg/dL)	0.99	0.96 – 1.02	0.607
Triglycerides (mg/dL)	0.99	0.98 – 1.00	0.242
25(OH)Vitamin D (≥20 mg/dL)	1.38	0.67 – 2.84	0.381

OR: odds ratio; CI: confidence interval; P < 0.05 was considered statistically significant

system (2). Unexpectedly, our study showed no associations between PSA and ferritin serum levels in both study populations. Indeed, we expected to find significantly higher ferritin concentrations in men with PSA "grey zone", according to studies reporting that high serum ferritin levels are positively associated with elevated PSA levels and PC risk (3). In the current research we did not observe any association between PSA levels and total cholesterol, LDL, HDL and triglycerides, respectively, either in men with PSA in "normal range" or in "grey zone". Our results are in line with a recent meta-analysis

Table III. *Logistic regression analysis assessing associations between examined variables and seasonal variability of 25(OH)Vitamin D ≥20 mg/dL*

Variables	OR	95% C.I.	P
Age (years)	0.96	0.89 – 1.03	0.238
BMI (kg/m^2)	1.03	0.89 – 1.19	0.674
PSA "grey zone"	1.46	0.70 – 3.05	0.309
Season (spring-summer)	2.16	1.03 – 4.53	0.042
Ferritin (µg/L)	1.00	0.99 – 1.01	0.556
Total cholesterol (mg/dL)	0.99	0.96 – 1.02	0.482
HDL (mg/dL)	1.03	0.98 – 1.07	0.240
LDL (mg/dL)	1.01	0.98 – 1.04	0.519
Triglycerides (mg/dL)	1.00	0.99 – 1.01	0.829

OR: odds ratio; CI: confidence interval; P < 0.05 was considered statistically significant

reporting no significant association between total cholesterol, HDL or LDL and risk of overall or high-grade PC (8). However, it has been reported that higher total cholesterol and LDL were significantly related to higher PSA levels and some studies also showed that obesity, hypertriglyceridemia and low levels of HDL are associated with reduced levels of PSA (9). We decided to focus on the relationship between 25(OH)Vitamin D and PSA since it has been thoroughly investigated lately. In the present study, no association was found between PSA and 25(OH) Vitamin D serum levels in men with PSA "grey zone" and in men with PSA < 4 ng/mL. Our results are in line with studies reporting no association of 25(OH)Vitamin D serum levels with PC incidence, even if controversial results have been showed on this topic: for example, a recent dose-response meta-analysis quantitatively examined the association of circulating 25(OH)Vitamin D concentration with PC and suggested that high 25(OH)Vitamin D levels are correlated with elevated risk of developing this malignancy (6, 10-11). In our study, the statistical analysis showed no significant differences in 25(OH) Vitamin D levels between men with PSA <4ng/mL and those with PSA ranging from 4 to 10 ng/mL, even if a larger percentage of men with sufficient levels (≥20 ng/mL) was found in the "grey zone" population (55.56%) than in the normal range one (44.44%). Additionally, the data obtained in our study indicated a significant seasonal variation of 25(OH)Vitamin D serum levels: in all men studied 25(OH)Vitamin D was higher in the spring-summer period than in the autumn and winter months, as reported in many investigations (12). The present study is of interest as, to the best of our knowledge, no previous research has explored any possible correlation between PSA "grey zone" and the biochemical parameters examined. We can speculate that we did not find any association between biochemical parameters and PSA levels because we studied men with PSA "grey zone" and no PC: future studies could be structured to investigate possible associations between biochemical parameters and PSA "grey zone" in men with a PC diagnosis and also to find correlations between biochemical parameters and tumor grading in this population. We are aware

that this research has some drawbacks. Firstly, a relatively small number of subjects constituted the two groups tested for each biochemical parameter selected. Further studies are needed to extend this work on larger cohorts to validate the results. Secondly, we focused only on certain biochemical parameters: it would be interesting to study also other analytes to better delineate PSA "grey zone" population. Finally, as a retrospective study we could not investigate a possible intake by the subjects of supplements containing 25(OH)Vitamin D: however, the seasonal variability of 25(OH)Vitamin D, which emerged from our study, greatly reduces the possibility that this dietary supplementation has occurred. Despite these limitations, this study clarifies some biochemical aspects of a slightly investigated population of men, which often are involved in invasive and unnecessary diagnostic procedures. In conclusion, our findings could represent a ground for additional researches concerning any potential association with biochemical profile of men with PSA "grey zone" and their risk of developing PC.

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