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C REACTIVE PROTEIN LEVEL AS A DIAGNOSE TEST FOR SLEEP APNEA-HYPOPNEA SYNDROME

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ABSTRACT. Sleep apnea-hypopnea syndrome is a disease characterized by repetitive episodes of partial or total collapse of the upper airways during sleep. The collapse of the upper airways and the repeated nocturnal desaturations of arterial blood in this disease lead to the development of chronic low-grade systemic inflammation, contributing to the pathogenesis of cardiovascular diseases. C-reactive protein is an inflammatory marker with pentameric structure that migrates electrophoretically in the gamma vicinity. The present study has aimed to determine the diagnostic ability of CRP levels for SAHS in order to facilitate exploratory tests in sleep medicine. In order to obtain a proper diagnostic test a compromise was made between sensitivity and specificity using Receiving Operating Characteristic curve. "Area under curve" was 0,7742 for a 95% confidence interval, p= < 0,0001. CRP values < 10 mg/ l showed a less sensitive test, but very specific. CRP values > 12 mg/ l showed a very sensitive test, but the specificity decreased dramatically. We have demonstrated that CRP levels cannot be used for positive diagnosis of SAHS, because, despite high values are associated with a positive diagnosis, the specificity of the test decreases dramatically when the CRP values are higher than 13 mg/ l.

Keywords: *apnea, hypopnea, polysomnography, C- reactive protein, the ROC curve*

INTRODUCTION

Sleep apnea-hypopnea syndrome (SAHS) is a disease that affects 4% of middle-aged men and 2% of middle-aged women [1].This disease is

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characterized by repetitive episodes of partial (hypopnea) or total (apnea) collapse of upper airways during sleep which arise from the pharyngeal muscle relaxation [2, 3, 4]. Along with sleep initiation, in these patients, a vicious cycle is repeated: upper airways obstruction, reopening them, a time for arousals and subsequent asphyxia [5]. All these events occurred every night, are associated with intermittent hypoxemia and hypocapnia, hyperventilation, and thus sleep fragmentation and excessive daytime sleepiness [2, 4].The collapse of the upper airways, repeated nocturnal desaturations of arterial blood, result in a chronic low-grade systemic inflammation, thereby contributing to the pathogenesis of atherosclerosis and the process of atheromatosis [6, 7].The C-reactive protein (CRP), interleukin 6, tumor necrosis factor alpha and pentraxin 3 are part of the inflammatory markers and cardiovascular pathologies encountered in respiratory disorders during sleep [1, 8]. Interleukin 6 is a pro-inflammatory cytokine and an inducer of the hepatic acute phase response which stimulates the production of CRP [1]. CRP is a non-glycosylated protein with pentameric structure that migrates electrophoretically in the gamma vicinity. This is an acute phase reactant that increases rapidly in response to tissue damage, viral/ bacterial and neoplastic pathologies. During tissue necrosis and inflammation resulting from microbial infection, the concentration of this protein may increase to 300 mg/ l in 12/ 24 h. At the same person, CRP concentration/ 24 h is almost stable, thus easy to detect a possible inflammatory response in patients with SAHS [9]. Fragmentation and sleep deprivation are pro-inflammatory by themselves. Meier- Ewert et al. demonstrated that 88 hours/ 12 days of sleep deprivation, or 10 days of sleep restriction for 4 hours/ night were associated with increased levels of CRP [10].

Validity of diagnostic procedures means its ability to identify subjects touched by the disease and healthy subjects. Establishing a diagnosis is an imperfect process, which can be expressed rather in terms of probability than in terms of certainty. A diagnostic test will not be effective unless the result is able to alter the probability of disease, or to reduce the likelihood of it to such a level, that treatment should not be done, or to rise to the level at which treatment is legitimate.

The probability of being touched by a disease is not fixed, but related to the diagnostic test: before the test, it is likely "a priori" (pre-test probability) - that is, simply, the prevalence; after the test, it is likely "a posteriori" (post-test probability) - is what is called the predictive value of the test.

The sensitivity of a test (or the likelihood of a positive real) is the probability of a positive test when there is disease. A sensitive test rarely misses the diagnosis in patients touched by the disease. False negative results are rare. Such a test should be chosen when a disease is serious and cannot be ignored, when there is a cure for the disease or when a possible false positive has harmful consequences for the patient. False negative results are rare, a sensitive test is useful for the doctor, especially when the result is negative; it can be pretty sure that eliminates the disease.

The specificity of a test (or negative real probability) is the probability of a negative test, when there is disease. A specific test rarely says that a subject is sick, when in reality it is not. False positive results are rare. A specific test should be chosen when a false positive can be harmful to the patient, in physical, psychological or economic terms. Since false positives are rare, a specific test is especially useful when the result is positive; the doctor can be pretty sure that the disease is present.

The compromise between sensitivity and specificity is achieved using a curve that characterizes the performance of the test (ROC Curve: Receiving Operating Characteristic). It is obvious that the doctor wants to order a diagnostic test at the same time, sensitive and specific. This is not possible in practice. You always have to make a compromise between sensitivity and specificity. ROC curve represents a way of expressing the relationship between sensitivity and specificity of a diagnostic test. This allows the description of test accuracy and can be used practically to compare two different tests to diagnose the same disease: accepted standard test (golden standard) and a new diagnostic test.

In these circumstances, the present study aims to determine the ability of CRP levels to diagnose SAHS in order to facilitate exploratory tests in sleep medicine.

RESULTS AND DISCUSSION

Of 100 patients entered into the study, 60 were diagnosed with SAHS. 55 patients had obstructive apnea- hypopnea. Forms of mild, moderate and severe disease were found in 2%, 21% and 37%. Like other studies in the medical literature, males predominated in 57% [11, 12]. 26 patients had values of CRP in the range of ≤ 1,4 to 9,52 mg/ l and 74 patients CRP values above 9,52 mg / l (Figure 1).

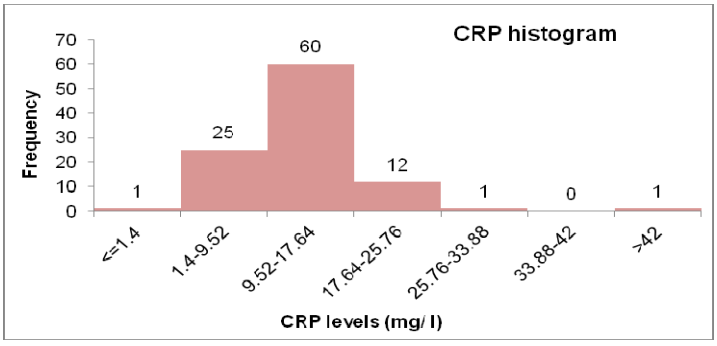

Figure 1. Distribution of patients after CRP levels

CRP Average in patients with SAHS was 14,54 and in those without apnea- hypopnea was 8, 62.

In this study, the moderate and severe forms of SAHS predominated, as the CRP levels presented in Figure 2. SAHS is a prevalent sleep disease and an independent risk factor in the development of cardiovascular diseases and in worsening of their morbidity and mortality. This was associated with risen levels of different circular and inflammatory markers [13]. There are studies that have demonstrated important and independent associations between the CRP levels and the severity of sleep apnea- hypopnea [2, 14, 15, 16]. Only a few studies showed an association between the moderate SAHS and high CRP level [17]. In our study the intermediary values of CRP were associated to the moderate forms of SAHS, as they were shown in figure 3. Nocturnal hypoxemia and sleep deprivation are often present in patients with SAHS and could be a way to mediate the association between SAHS and increased levels of inflammatory markers. [18, 19] Oxygen desaturation index could explain some of the apnea- hypopnea index (AHI) associations with the CRP level. [19]

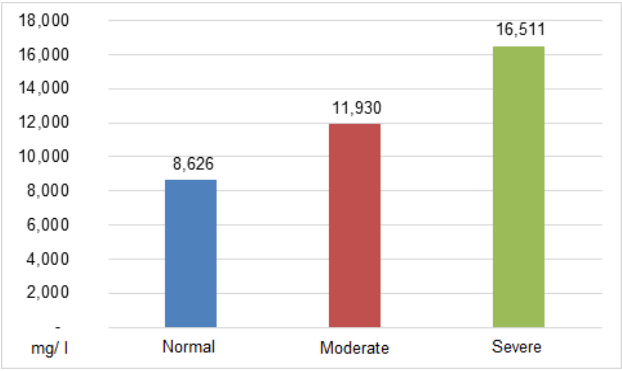

Figure 2. Distribution of CRP levels according to the severity of SAHS

The ability of CRP to diagnose SAHS was performed using ROC analysis and showed an "area under curve" of 0,7742 for a confidence interval of 95% (p = <0,0001); table 1. In figure 3 we showed the ROC curve of CRP.

Table 1. ROC curve for CRP vs SAHS

Area under the ROC curve	
Area	0,7742
Std. Error	0,04696
95% confidence interval	0.6821 to 0.8662
P value	< 0.0001
Data	
Control	40
Patient	60

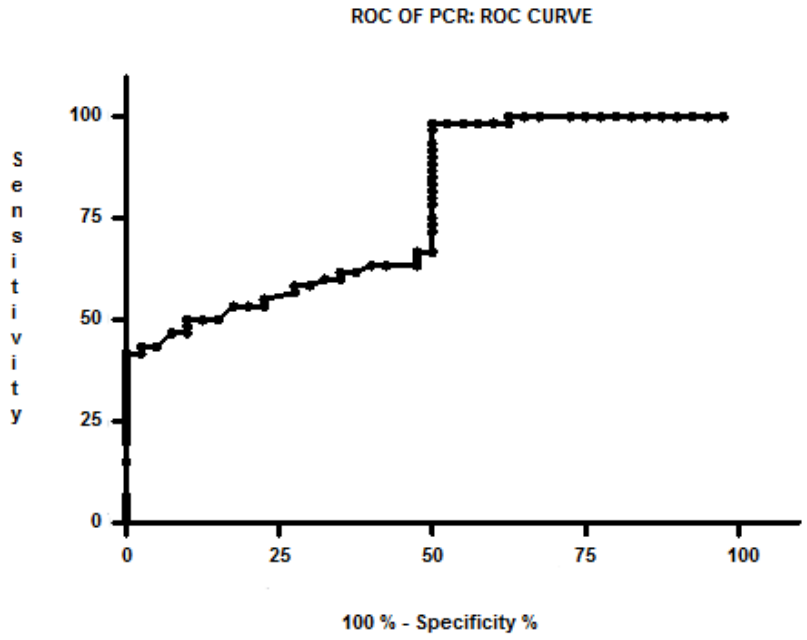

Figure 3. Graphical representation of the ROC curve

CRP values <10 mg/ l showed that the test was less sensible, there are many false- negative patients, but was very specific, so that all patients with CRP levels below 10 mg/ l were correctly diagnosed as not having SAHS.

CRP values > 12 mg/ l have shown that the test was very sensitive, being detected all patients with SAHS, but specificity decreased dramatically, leading to errors in the classification of subjects in false positive patients.

CONCLUSIONS

We have demonstrated that CRP levels can be used as a negative diagnostic test for SAHS, this eliminating the suspicions of sleep apnea-hypopnea because it is a quick exploration, cheap, easy and practiced in routine.

CRP levels cannot be used for positive diagnosis because despite increased values are associated with a positive diagnosis, the specificity of the test decreases dramatically to more than 13 mg/ l of the CRP.

EXPERIMENTAL SECTION

Patients and study design

This study was conducted for 6 months in Sleep Laboratory Alpes-Leman in Contamine- sur Arve, France. We included patients aged between 18 and 80 years, 60 subjects with a positive diagnosis of SAHS (if after performing the polysomnography (PSG) the AHI ≥ 5/ hour of sleep) and 40 subjects as control group with negative diagnosis of SAHS (AHI < 5/ h). For each patient we included demographic data, levels of CRP and polysomnographic data. CRP values ≥ 10 mg/ l were considered pathological. Patients with various bacterial or viral infections, inflammatory disease/ different systemic or malignant neoplasia were excluded. The study was approved by the chief of Pneumology, being consistent with the principles of the Declaration of Helsinki. Each patient gave his verbal consent to participate in research. There weren't used experimental methods on subjects and the tests applied were the ones current of the hospital, the purpose of the paper was to emphasize the standard methods of diagnosis.

Polysomnographic monitoring

The polysomnography was performed with "Morpheus hand held", recorded by Micromed s.p.A connected to System Plus Evolution 1061. This unit has recorded various nocturnal signals such as: electro-encephalogram, electro- oculogram, electro- cardiogram, nasal respiratory flow, pulse, oxygen saturation, snoring intensity, mentonier tone, thoracic and abdominal respiratory movements, leg movements, the position of the body and the time spent in bed. PSG recordings were performed manually using the program "Rembrandt Analysis Manager 7.5", by doctors attached to the service, in accordance with the recommendations of Rechtschaffen – Kales and the American Academy of Sleep Medicine.

An AHI ≥ 5/ h of sleep was needed to diagnose SAHS, an AHI ≥ 5 < 15 indicated mild SAHS and AHI ≥ 15/ h indicated a moderate to severe SAHS. PSG recordings were not taken into account if the total sleep time was < 180 minutes or quality of the main signals (electro- encephalogram, oxygen saturation, nasal flow, toraco- abdominal movements) was lower than 20% of the total registration.

Measurement of C- reactive protein

The amount of CRP in the serum was dispensed obtained after centrifugation (3000 revolutions/ minute, during 10 minutes) of the patient's peripheral venous blood. CRP concentration was measured by latex

turbidimetry. Latex- turbidimetry was based on antigen- antibody complex formation in solution. The solutions of antigen and antibody were mixed, being required small quantities of reactive; aggregate formation occurring rapidly. This method was based upon the reactions between C- reactive protein and latex covalently bound antibodies against human CRP. CRP values were determined photometrically.

Statistical Analysis

It was performed a case- control study between CRP concentrations and the presence or absence of apnea- hypopnea. Qualitative variables were described using frequency tables, contingency and column graphs. To describe quantitative variables we used the average (mean), median, range interquartilic Q25, Q75, frequency tables, histograms. Test "t" Student for independent samples or analysis of variance ANOVA were applied for comparison of means. Mann-Whitney/ Kruschal- Wallis tests were used for a confidence interval of 95%. We made the ROC curves and determined the "area under the curve" for a confidence interval of 95%. We calculated the sensitivity and specificity of CRP for the diagnosis of SAHS. In order to calculate the ROC curve and its parameters it was used specific software named GraphPad Prism.

ABREVIATIONS

AHI, Apnea- Hypopnea Index; CRP, C- Reactive Protein; PSG, Polysomnography; ROC, Receiver Operating Characteristic; SAHS, Sleep Apnea-Hypopnea Syndrome.

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