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Screening for raised base-line values of serum CRP as an independent risk factor for cardiovascular disease among adults in Bethlehem District using hs-CRP assay.

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Abstract

C-reactive protein is a non-specific indicator of inflammation. The CRP test is useful in evaluating patients with an acute myocardial infarction (AMI). The level of CRP correlates with peak levels of the CK-MB isoenzyme, but CRP peaks occur 18 to 72 hours later. Failure of CRP to normalize may indicate ongoing damage to the heart tissue. Levels are not elevated in patients with angina. Although CRP assays only report levels > 3 mg/L, the hs-CRP assay reports levels as low as 0.1 mg/L, and is thus used in cardiovascular disease (CVD) risk stratification. Patients with hs-CRP levels < 1 mg/L are categorized as having lower relative risk for cardiovascular events. Those with levels of 1 to 3 mg/L are at intermediate risk, and those with levels > 3 mg/L are at higher relative risk. The purpose of this study is to screen for raised base-line serum CRP levels among adults from Bethlehem District using the CRP-ultrasensitive assay (Micro CRP/Ultra CRP Latex turbidimetry). A total of 189 serum samples were obtained from patients doing various tests in different laboratories from Bethlehem District and were tested for baseline values of CRP using hs-CRP assay (CRP-ultrasensitive/Latex turbidimetry). All patients had normal ESR values to exclude the presence of inflammation. Approximately 17.5% of Bethlehem district adults in the age range of 18-83 have hs-CRP levels >3.0 mg/L and therefore can be stratified as having high risk while

more than half of that population (56.1%) have hs-CRP levels <1.0 mg/L.

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Patients with average risk (26.5%) are recommended to monitor their cholesterol, triglycerides, blood pressure, and hs-CRP value on a regular basis since the presence of other risk factors increases their chance of having CVD. The highest percentage of high cardiac disease risk (24.2%) was found in patients in their 40's and should therefore actively control any other CVD risk factor and could start taking statins based on their hs-CRP value therefore it is hoped that clinicians realize the benefit of adding hs-CRP assay to their battery of CVD markers.

Introduction

Inflammation is pivotal in all phases of atherosclerosis. Among the numerous inflammatory biomarkers, the largest amount of published data
supports a role for C-reactive protein (CRP) as a robust and independent risk marker in the prediction of primary and secondary adverse cardiovascular events. In addition to being a

risk marker, there is much evidence indicating that CRP may indeed participate in atherogenesis[1].

CRP, named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae*, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage[2].

CRP belongs to the pentraxin family of calcium dependent ligand-binding plasma proteins, in humans the other member is serum amyloid P component (SAP). The human CRP molecule (Mr 115,135) is composed of five identical nonglycosylated polypeptide subunits (Mr 23,027), each containing 206 amino acid residues. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry[2] (Figure 1). The CRP gene has been mapped to chromosome 1[1].

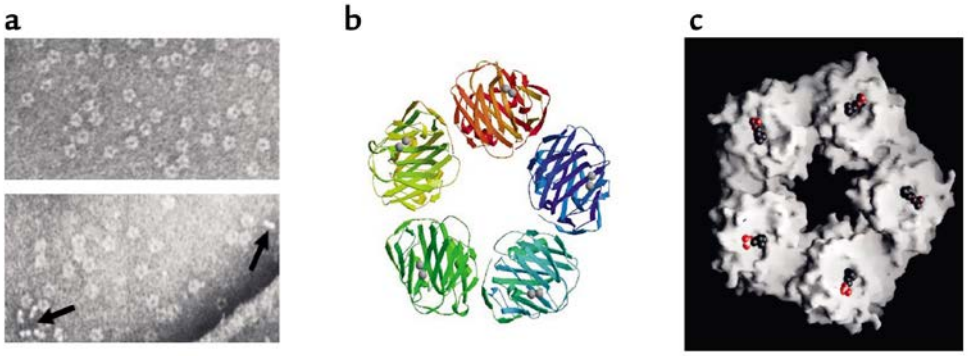

Figure 1. Molecular structure and morphology of human CRP. (**a**) Negatively stained electron micrograph showing the typical pentameric disc-like structure face-on and side-on (arrows). (**b**) Ribbon diagram of the crystal structure, showing the lectin fold and the two calcium atoms (spheres) in the ligand-binding site of each protomer (6). (**c**) Space-filling model of the CRP molecule, showing a single phosphocholine molecule located in the ligand-binding site of each protomer[2].

The plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so that the sole determinant of circulating CRP concentration is the synthesis rate, which thus directly reflects the intensity of the pathological processes stimulating CRP production[(2)]. The process of phagocytosis results in macrophage activation, which leads to the secretion of cytokines such as IL-6 which stimulates hepatocytes to secrete CRP that is deposited on some bacteria and functions as an opsonin[(3)]. To date, in phagocytes, it has been shown to bind Fcγ receptor (FcγR) I and II, and its function appears to clear apoptotic and necrotic cells[(1)].

The CRP test is useful in evaluating patients with an acute myocardial infarction (AMI). The level of CRP correlates with peak levels of the MB isoenzyme of creatine kinase, but CRP peaks occur 18 to 72 hours later. Failure of CRP to normalize may indicate ongoing damage to the heart tissue. Levels are not elevated in patients with angina[(4)].

The recent development of a high sensitivity assay for CRP (hs-CRP) has enabled accurate assays at even low levels. Atheromatous plaques in diseased arteries typically contain inflammatory cells. Multiple prospective studies have also demonstrated that baseline CRP is a good marker of future cardiovascular events. The CRP level is a strong predictor of cardiovascular events than the LDL-cholesterol level. However, when used together with the lipid profile, it adds prognostic information to that conveyed by the Framingham risk score. In patients with stable coronary artery syndromes, hs-CRP measurement may be useful as an independent marker for assessing likelihood of recurrent events, including death, myocardial infarction (MI), or restenosis after percutaneous coronary intervention (PCI)[(4)].

The concentration of CRP increases 4 to 6 hours after acute tissue injury or inflammation and declines rapidly with resolution of the injurious process. In healthy persons, normal CRP levels are generally considered to be < 3 mg/L. Low-grade inflammation can produce minor elevations of CRP in the 3- to 10-mg/L range. C-reactive protein levels >10 mg/L may suggest the presence of an underlying inflammatory disease, although levels in this range can be seen on a genetic basis in apparently healthy individuals[5]. Although CRP assays only report levels > 3 mg/L, the hs-CRP assay reports levels as low as 0.1 mg/L, and is thus used in CVD risk stratification. Patients with hs-CRP levels < 1 mg/L are categorized as having lower relative risk for cardiovascular events. Those with levels of 1 to 3 mg/L are at intermediate risk, and those with levels > 3 mg/L are at higher relative risk[5] (Table 1).

Table 1: Cardiovascular Risk Stratification by hs-CRP Value.[5]

hs-CRP, mg/L	**Cardiovascular Risk**
< 1	Low
1–3	Intermediate/average
> 3	High

Cholesterol screening has been used as a tool to identify individuals who are at increased risk of developing future coronary events. Although this approach has been useful, it fails to identify almost one-half of the 1.3 million individuals who develop MI in the US each year who have either normal or only moderately increased serum cholesterol concentration[6]. In the most recent comprehensive meta-analysis of > 50 prospective studies, hs-CRP was consistently found to be an independent predictor of CVD. In fact, in a 2010 meta-analysis, the magnitude

of risk associated with hs-CRP was found to be greater than that associated with either blood pressure or cholesterol[5] (Table 2).

Table 2 : Magnitude of Independent Risk Associated with hs-CRP Compared with Blood Pressure and Cholesterol.[5]

Factor	Risk Ratio (95% CI)
hs-CRP	1.37 (1.27–1.48)
Systolic blood pressure	1.35 (1.25–1.45)
Non–HDL-C	1.28 (1.16–1.40)

Data from Emerging Risk Factors Collaboration. **Abbreviations:** CI, confidence interval; CRP, C-reactive protein; hs-CRP, high-sensitivity CRP; non–HDL-C, non–high-density lipoprotein cholesterol[5].

It has been suggested that the addition of hs-CRP level to the Framingham risk score can provide additional prognostic information. It has been reported that women aged > 65 years with an hs-CRP > 3 mg/L and a Framingham 10-year risk of > 20% had a 31% incidence of coronary heart disease versus a 16% incidence in women in the group with a normal hs-CRP. Men aged > 65 years in the 10% to 20% and > 20% Framingham risk ranges were also at higher risk of coronary heart disease if they had an hs-CRP > 3 mg/L[5].

Although high-sensitivity C-reactive protein (hs-CRP) has emerged as a cardiovascular marker, questions arise regarding the relative information provided by other inflammatory molecules. Correlations were significant between hs-CRP

concentrations and leukocyte and platelet counts, as well as haptoglobin, orosomucoid, Interleukin-6 (IL-6), and intercellular adhesion molecule-1 (ICAM-1) concentrations ($p \leq 0.001$). Correlation coefficients for ICAM-1 were higher in men than in women ($p \leq 0.05$). When stratifying subjects according to hs-CRP levels, the group with high hs-CRP levels had significantly higher haptoglobin and orosomucoid concentrations than the others, in addition to higher leukocyte counts and IL-6 concentrations in women, and platelet counts and ICAM-1 concentrations in men[7].

It has been shown that local chronic inflammation may lead to colorectal carcinogenesis via adenomatous polyps. Serum levels of interleukin-6, C-reactive protein and carcinoembryonic antigen were significantly higher in cancer patients when compared to adenoma patients and healthy subjects, and increased in more advanced stages of disease and in patients with nonresectable tumors. Based on Cox's analysis, the elevated preoperative serum level of C-reactive protein was an independent significant prognostic factor for patients' survival[8].

CRP is clearly a risk marker for cardiovascular disease and is recommended for use in primary prevention. In addition, CRP appears also to contribute to atherogenesis as shown by some of its proatherogenic effects on endothelial cells [increased vascular cell adhesion molecule (VCAM), ICAM-1, E-selectin, monocyte chemotactic protein-1 (MCP-1), monocyte adhesion], on monocyte-macrohpages (increased tissue factor, increased superoxide and myeloperoxidase, and promoted OxLDL uptake and decreased cholesterol efflux), and on smooth muscle cells [increased antithrombin-1 (AT-1) and vascular smooth muscle cell (VSMC) migration and proliferation, increased inducible nitric oxide (iNOS) and tissue factor][1]. Knowledge of the structure and function of CRP-including its three-dimensional structure alone

and complexed with ligands-coupled with experience in developing an inhibitor of the related protein SAP establishes an excellent platform for drug design[2].

Statins are known to decrease hs-CRP levels in a manner largely unrelated to the reduction of low-density lipoprotein cholesterol (LDL-C). In patients with acute coronary syndromes, those with an hs-CRP < 2 mg/L after statin therapy had a better prognosis than those with higher hs-CRP levels, even when LDL-C was reduced to < 70 mg/dL[5].

A variety of methods are employed by the various manufacturers of hs-CRP assays. These include several immunoturbidimetric approaches chemiluminescence, particle-enhanced immunonephelometry, and the lateral flow immunoassay technique of the Cholestech LDX hs-CRP assay[9]. The agreement between the Dade Behring and Olympus methods for relative risk class assignments is 95.4%. Statistical analysis of the agreement between the two methods for each relative risk class showed that the differences between the methods are not statistically significant ($p > 0.10$)[10]. The i-CHROMAk hs-CRP assay system is comparable to those of other well-known fully automated hs-CRP assay and is suitable for point-of-care testing (POCT) in detection and quantification of hs-CRP[11]. The purposes of this study are to screen for raised base-line serum CRP levels among adults from Bethlehem District and to stratify patient risk using the CRP-ultrasensitive assay (Micro CRP/Ultra CRP Latex turbidimetry). The presence of raised base-line serum CRP levels greater than 3.0 mg/L is considered a risk factor for coronary artery disease. So, this study will help us assess the risk of developing cardiovascular disease or other processes involving inflammation among adults from Bethlehem District.

Materials and Methods:

A total of 189 serum samples were obtained from patients doing various tests in different laboratories from Bethlehem District and were tested for baseline values of CRP using hs-CRP assay (CRP-ultrasensitive/Latex turbidimetry). The patient ages ranged from 18-83 years old and had normal erythrocyte sedimentation rate (ESR) values to exclude the presence of inflammation or infection which elevates CRP values above 6 mg/L. Any value below 6 mg/L is considered normal and is subject to hs-CRP testing. The effect of factors other than inflammation on CRP value is trivial and negligible. The CRP-ultrasensitive is a quantitative turbidimetric test for the measurement of low levels of CRP in human serum and plasma. Latex particles coated with specific anti-human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known concentration. A control was run to monitor the performance of the assay procedure. The CRP ultrasensitive has a linearity of up to 5 mg/L.

Results:

Patients were stratified into low, average, and high risk for coronary artery disease based on CRP results in mg/L: Low risk <1.0 mg/L, Average risk: 1.0-3.0 mg/L, and High risk: >3.0 mg/L with a set upper limit of 5.0 mg/L. The results are shown in tables 3 and 4, figures 2 and 3 below:

Table 3: Cardiovascular risk stratification by hs-CRP Value.

Cardiac Risk Level	Number of Patients	Cardiac Risk Frequency

Low risk	106	56.1%
Average risk	50	26.5%
High risk	**33**	**17.5%**

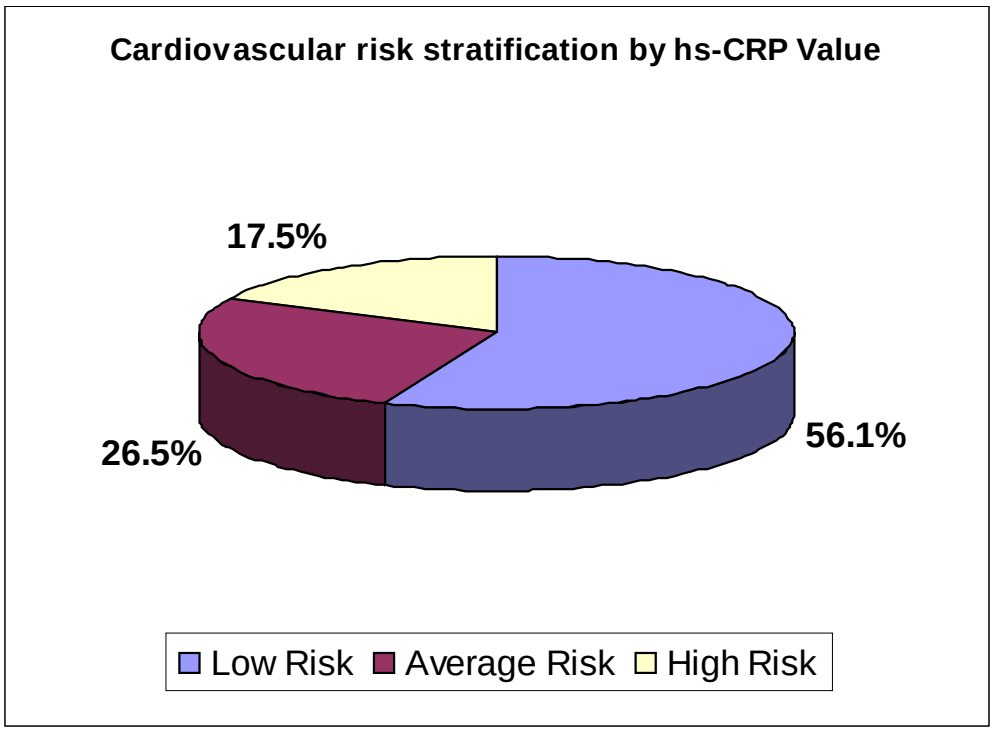

Figure 2: Cardiovascular risk stratification by hs-CRP value.

Table 4: The frequency of high risk patients based on age distribution.

Age Distribution (Years)	Number of Patients	High Risk Frequency
20-29	2	6.1%
30-39	6	18.2%
40-49	**8**	**24.2%**
50-59	6	18.2%
60-69	6	18.2%
70-79	2	6.1%
80-89	3	9.1%

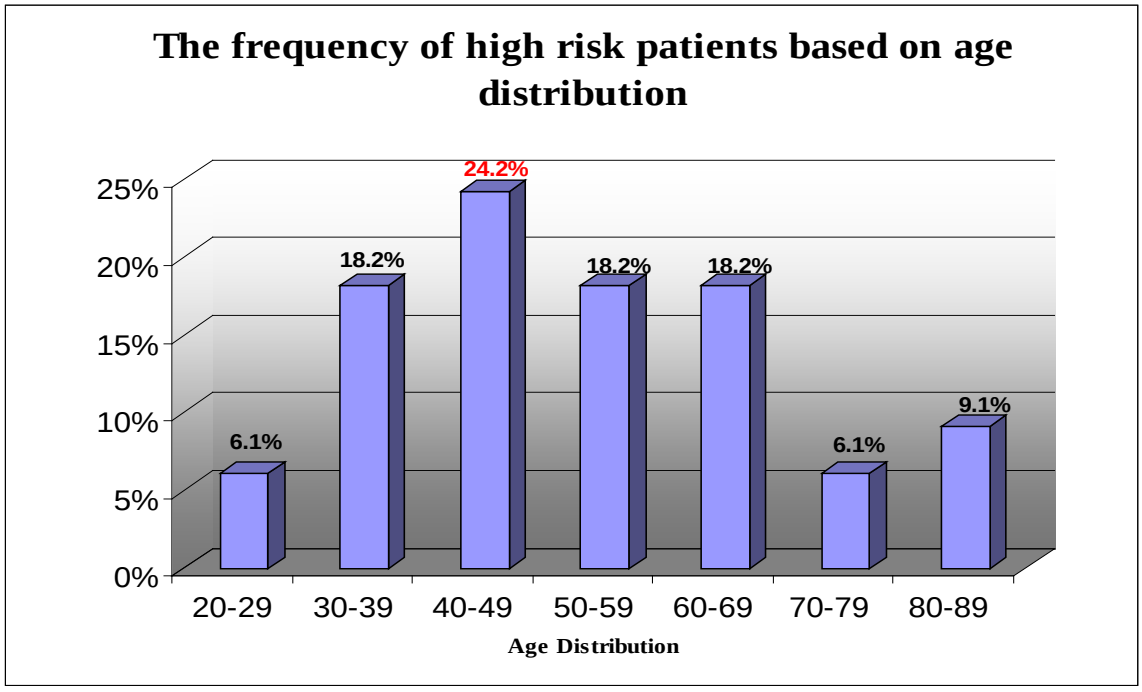

Figure 3: The frequency of high risk patients based on age distribution

Discussion:

Because CRP is an acute-phase reactant, there can be intra-individual variability, although several studies demonstrate that the stability of hs-CRP over time is quite similar to that of cholesterol and blood pressure[(5)]. It is therefore recommended that the average of 2 separate hs-CRP measurements, taken at least 2 weeks apart, be used when estimating risk or prognosis based on hs-CRP[(5)].

Approximately half of US adults aged > 55 years have hs-CRP levels > 2 mg/L, and in most population studies, 95% have levels < 10 mg/L. Levels of hs-CRP vary modestly by race. Data from the Dallas Heart Study demonstrated that black women had higher median hs-CRP levels than black men or white men and women. Black men had higher median hs-CRP levels than white men[(5)].

C-reactive protein levels > 10 mg/L are often associated with infection, some inflammatory diseases, or malignancy; some patients have levels in this range on a purely genetic basis. For example, tissue trauma or necrosis associated with surgery, burns, myocardial infarction (MI), or pancreatitis can also produce high CRP levels. In these cases, an elevated erythrocyte sedimentation rate (ESR) is often noted in association with high CRP. Analysis of the Framingham Offspring Study participants who were free of CVD showed that inflammatory conditions were present in > 40% of the men and women with CRP values > 10 mg/L. C-reactive protein levels > 10 mg/L doubled the likelihood of the presence of an inflammatory condition compared with the risk for participants with hs-CRP levels < 1

mg/L; however, such patients were often found to have quite a high vascular risk as well[5].

The Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) released a joint statement in 2003 on the clinical use of biomarkers in CVD risk assessment, estimation of prognosis, and management decisions. Key recommendations with class 2a evidence (ie, conflicting evidence/opinion, weight in favor of usefulness/efficacy) included[5]:

hs-CRP is the CVD inflammatory marker of choice for clinical practice;
patients with an intermediate (10%–20%) risk of CVD may benefit from measurement of hs-CRP for evaluation and therapy decisions; and hs-CRP may be useful for estimating prognosis in patients with CVD (including death and recurrent events).

Approximately 17.5% of Bethlehem district adults in the age range of 18-83 have hs-CRP levels >3.0 mg/L and therefore stratified as having high risk while more than half of that population (56.1%) have hs-CRP levels <1.0 mg/L. Patients with average risk (26.5%) are recommended to monitor their cholesterol, triglycerides, blood pressure, and hs-CRP value on a regular basis since the presence of other risk factors increases their chance of having CVD. The highest percentage of high cardiac disease risk (24.2%) was found in patients in their 40’s and should therefore actively control any other CVD risk factor. If for example they have elevated LDL-cholesterol levels, they should take statins and/or change their lifestyle depending on the level of cholesterol elevation in the blood. Patients could start taking statins based on their hs-CRP value. Clinicians should encourage patients with high risk baseline CRP values to cease smoking. Therefore it is hoped that they realize the benefit of

adding hs-CRP assay to their battery of CVD markers. A large prospective study covering the West Bank is needed in the future to better assess the risk of CVD in the Palestinian Population. This future study would also stratify patients risk not only according to age but also to gender.

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