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Study protocol

Effects of chronic inflammatory bowel diseases on left ventricular structure and function: a study protocol

Ugo Cioffi*[1], Michele M Ciulla[2], Matilde De Simone[1], Roberta Paliotti[2], Alberto Pierini[2], Fabio Magrini[2], Fiorenzo Botti[1] and Ettore Contessini-Avesani[1]

Address: [1]Department of Surgery, University of Milan, Ospedale Maggiore di Milano – IRCCS, Via F Sforza, 35 – 20122 – Milano, Italy and [2]Istituto di Clinica Medica Generale e Terapia Medica, Centro di Fisiologia Clinica e Ipertensione, University of Milan, Ospedale Maggiore di Milano – IRCCS, Via F Sforza, 35 – 20122 – Milano, Italy

E-mail: Ugo Cioffi* - ugo.cioffi@unimi.it; Michele M Ciulla - img.lab@mailserver.unimi.it; Matilde De Simone - matilde.desimone@unimi.it; Roberta Paliotti - roberta.paliotti@unimi.it; Alberto Pierini - bert.g@tiscali.it; Fabio Magrini - fabio.magrini@unimi.it; Fiorenzo Botti - fiorenzo.botti@unimi.it; Ettore Contessini-Avesani - ettore.contessini@unimi.it

*Corresponding author

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Abstract

Background: Experimental evidences suggest an increased collagen deposition in inflammatory bowel diseases (IBD). In particular, large amounts of collagen type I, III and V have been described and correlated to the development of intestinal fibrotic lesions. No information has been available until now about the possible increased collagen deposition far from the main target organ. In the hypothesis that chronic inflammation and increased collagen metabolism are reflected also in the systemic circulation, we aimed this study to evaluate the effects on left ventricular wall structure by assessing splancnic and systemic collagen metabolism (procollagen III assay), deposition (ultrasonic tissue characterization), and cardiac function (echocardiography) in patients with different long standing history of IBD, before and after surgery.

Methods: Thirty patients affected by active IBD, 15 with Crohn and 15 with Ulcerative Colitis, submitted to surgery will be enrolled in the study in a double blind fashion. They will be studied before the surgical operation and 6, 12 months after surgery. A control group of 15 healthy age and gender-matched subjects will also be studied. At each interval blood samples will be collected in order to assess the collagen metabolism; a transthoracic echocardiogram will be recorded for the subsequent determination of cardiac function and collagen deposition.

Discussion: From this study protocol we expect additional information about the association between IBD and cardiovascular disorders; in particular to address the question if chronic inflammation, through the altered collagen metabolism, could affect left ventricular structure and function in a manner directly related to the estimated duration of the disease.

Background

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) of unknown origin and pathogenesis of adolescent and young adulthood, with

peak incidence occurring between 15 and 30 years; approximately 25% of patients are diagnosed during the first two decades of life. These conditions are characterized by focal or diffuse inflammation of the alimentary tract [1–4]. The chronic inflammatory status and the need of a longstanding therapy expose the patient to several extraintestinal manifestations. Cardiovascular ones are not rare findings and include autoimmune manifestations such as Takayasu's arteritis [5,6], pericarditis, myocardiopericarditis [7], drug-induced effects such as steroid-hypertension [8], pericarditis related to long-standing mesalazine treatment [9], thrombosis of the deep veins of the legs, and pulmonary embolism [10]. Chronic inflammation is also associated with an increase in collagen deposition to the main target organ; in particular the development of intestinal fibrotic lesions containing large amounts of collagen type I, III and V has been described [11,12]. Until now to our knowledge no reports are available in literature about the possible increased collagen deposition far from the main target organ in IBD. However, the cardiac involvement during inflammatory diseases such as systemic lupus erythematosus (13), systemic sclerosis (14), ankylosing spondylitis (15), and rheumathoid arthritis (16) has been described and ascribed to myocardial fibrosis.

This perspective study has the objective to determine the effects of chronic inflammation on left ventricular wall by assessing collagen metabolism, deposition, and cardiac function in patients with different long standing history of IBD, before and after surgery.

Methods/Design

Two teams will participate to the study: General Surgeons and Cardiologists. All data will be recorded in a database.

Study population

Thirty patients affected by active IBD, 15 with Crohn and 15 with Ulcerative Colitis, submitted to surgery will be enrolled in the study in a double blind fashion; they will be studied before the surgical operation and 6, 12 months after surgery. Disease activity will be assessed according to the Crohn's Disease Activity Index (17) and the Truelove-Witts index (18) for Crohn and ulcerative colitis, respectively. A control group of 15 healty age and gender-matched subjects will be also studied. At each interval blood samples will be collected in order to assess the collagen metabolism; a transthoracic echocardiogram will be recorded for the subsequent determination of cardiac function and collagen deposition.

Collagen metabolism (PIIIP)

Different kinds of collagens have been identified in humans; all of them derive from longer precursor molecules (procollagens), that are synthesized intracellulary and secreted in extracellular space where they are cleaved by aminoproteases [19,20]. Among the different kinds of precursors, type III is one of the most abundant interstitial procollagens in myocardium. Since its aminoterminal propeptide (PIIIP) is formed in equimolar proportions to collagen, serum measurements of this fragment can provide an index of collagen synthesis, also during acromegaly [21]. Concerning IBD, other Authors have described changes in PIIIP in patients with active Crohn's disease, before and after specific medical therapy [11,12].

At baseline after an overnight fast, before surgery (systemic PIIIP) and during surgery (splancnic PIIIP), serum levels of PIIIP will be assessed by commercial radioimmunoassay (Orion Diagnostics, Finland) which sensibility, intra-assay variation and inter-assay variation are respectively: 2,6 mcg/l, 4,0% and 4,3%. Normal ranges of PIIIP concentrations are $3,1 \pm 1,1$ mg/l. Following the same procedure, blood samples will be collected during the follow-up, to assess serum PIIIP concentrations 6 and 12 months after surgery.

Left ventricular function

Standard M-mode, two-dimensional and pulsed Doppler echocardiographic studies (Acuson 128XP; Acuson Inc., Mountain View, California – USA) will be performed, before the surgical operation and 6, 12 months after surgery, in left lateral recumbent position after a 10 minutes resting period, according to the reccomandations of the American Society of Echocardiography [22]. The following measurements will be recorded on M-mode tracing: left ventricular (LV) end-diastolic and end-systolic diameters, left atrial end-systolic diameter, interventricular septum thickness (IVST), posterior wall thickness. The relative thickness of the LV wall, with respect to the LV end-diastolic diameter, will be also calculated. The LV myocardial mass will be calculated using Deveraux's formula from the M-mode measurements [23]. LV hypertrophy will be considered when LV myocardial mass values corrected for body surface area (LVMI) is greater than or equal to 125 g/m^2 in males and females, according to the Penn Convention sex independent criteria. The Doppler study will provide indexes of left ventricular filling, which will be derived from the mitral flow velocities curves: maximal early diastolic flow velocity (E, cm/sec), maximal late diastolic flow velocity (A, cm/sec) and the E/A ratio (normal value > 1). Finally, ejection fraction (EF%), that is an index of systolic ventricular function, will be also calculated.

The sonographer will be blinded to all clinical and metabolic data.

Collagen deposition (UTC)

Experimental and clinical studies have demonstrated the possibility of non invasively assessing the ultrastructural

properties of tissues by ultrasound. In particular, interstitial collagen, because of its acoustic impedance, has been proven to be the main determinant for the echo texture image from the myocardium [24,25], and quantitative analysis of the ultrasound signals from the myocardium has demonstrated increased sensitivity for detection of collagen accumulation [26–28].

Echocardiographic images and simultaneous ECG tracings will be recorded on videotape in S-VHS format. Two representative subsequent cardiac cycles in each subject (synus rhythm at the surface electrocardiogram) will be digitized off-line from the videotape onto a personal computer (Power Machintosh G3 av, 300 MHz, 136 MB RAM, 8 GB hard drive; Apple Computer, Inc., Cupertino, California – USA) for the subsequent echoreflectivity analysis, using the built-in video digitizer card operating at 30 frames/sec and 8 bits/pixel on a standard PAL pixel matrix (640 × 480 pixels).

In order to obtain a better visualisation of the myocardial structure, the colour scale will be set at 256 colours (0 = yellow, 128 = magenta red, 255 = black), re-coding the original 256 echo grey scale (0 = white, 255 = black). UTC will be performed by two operators, blinded to all clinical and metabolic data, using two-dimensional long-axis end-diastolic echo recorded images. In each patient the region of ventricular wall for the analysis will be selected by positioning a square selection tool (5 × 5 mm) in the mid portion of interventricular septum, avoiding areas of echo drop-out. From each selection, according to a previously described procedure [29], a colour-level histogram representing the frequency distribution (number of pixels versus echo intensity) will be derived, using a software developed in our laboratory. Histograms will be described in terms of average pixel intensity (mCS) and colour spectrum width (broad band, Bb); the latter indicates the spread of the echoes about the mean distribution and reflects the interactions of echoes within the tissue, as a function of ultrasound velocity and distance between scatters. For each patient we will consider the average of three consecutive end-diastolic measurements. Collagen content (derived collagen volume fraction, dCVF%; normal values up to 2%) will be predicted as a dependent variable in a regression model using the independent variable Bb as predictor according to the following formula: dCVF% = (Bb – 43,4) / 22,67. Bb, in fact, has been proven to correlate with the histologically assessed collagen content in a previous study [29]. To avoid the effects on image texture caused by the characteristics of the ultrasound image system, all mCS values obtained from the interventricular septum will be normalized for blood echoreflectivity (black = 255), assessed along the same axis inside the left ventricle, in each single patient (Figure 1).

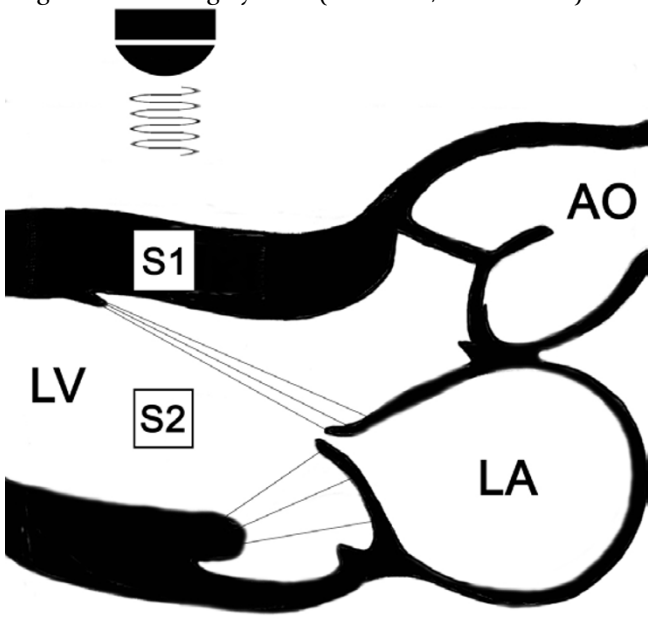

Figure 1
Schematic representation of a 2D long-axis echography of the heart illustrating the region of analysis (S1) in the mid-apex interventricular septum from which the measurements will be taken. Values obtained will be normalized for blood echoreflectivity assessed along the same axis inside the left ventricle (S2). LV = Left Ventricle; LA = Left Atrium; AO = Aorta. S1 and S2 indicate the regions of interest for the ultrasonic tissue characterization (myocardium and blood, respectively).

Statistical analysis

Data will be analyzed using a computer statistical software (SPSS – Rel 10; SPSS Inc., Chicago, Ill). All the quantitative variables will be tested for Gaussian distribution with the Kolmogorov-Smirnov test; all of them that will follow this distribution will be presented as mean ± standard deviation.

Differences at baseline in collagen parameters between IBD patients and controls will be tested for significance using the analysis of variance with the Bonferroni correction. The relation between collagen parameters and the estimated duration of the disease will be expressed in terms of regression analysis. The intra-assay variability will be tested in 10 randomly chosen subjects by repeating all the collagen determinations in 3 different samples in each subject. Interobserver and intraobserver reproducibility of UTC measurements will be evaluated by comparing measurements made by two different observers on the same day and by the same observer in two different days, respectively. To evaluate changes over time (before and after surgery) in collagen parameters in each patient, MANCOVA for repeated measurements will be used; age and sex

will be included as covariates in the analysis. In all cases a p value less than 0.05 will be considered significant.

Discussion

Till now the link between IBD and cardiovascular diseases has been reported only in few published cases, but none of them has focused the attention on the possible increased collagen deposition far from the main target organ. Because increased myocardial collagen deposition is a recognized marker of pathologic structural remodelling, at this respect, from this study protocol we expect additional information about what happens at the heart level after a long standing history of IBD and to elucidate wheather or not the surgical option could arrest an evenctual cardiac damage.

List of Abbreviations used

Inflammatory bowel diseases = IBD

Left ventricle = LV

Interventricular septum thickness = IVST

Left Ventricular Mass Index = LVMI

Transmitral E/A ratio = E/A

Ejection fraction = EF%

Ultrasonic Tissue Characterization = UTC

Average pixel intensity = mCS

Broad band = Bb

Derived Collagen Volume Fraction = dCVF%

Procollagen III propeptide = PIIIP

Competing interests

None declared

Authors' contributions

UC will enroll and follow-up the patients for the clinical and surgical aspects; he participated in the design of the study; MMC will perform the videodensitometric analysis of echocardiographic images; he participated in the design of the study; MDS will enroll and follow-up the patients for the clinical and surgical aspects; she drafted the manuscript; RP will perform the statistical analyses; she drafted the manuscript; AP will perform echocardiograms; FB will enroll and follow-up the patients for the clinical and surgical aspects; FM participated in the design and coordination of the study; ECA participated in the design and coordination of the study.

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