Digestive and Liver Disease 47 (2015) 702–708

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Adrenal function and microbial DNA in noninfected cirrhotic patients with ascites: Relationship and effect on survival

Alessandro Risso[a,*], Carlo Alessandria[a], Lavinia Mezzabotta[a], Chiara Elia[a], Alida Andrealli[a], Maurizio Spandre[a], Paola Di Luigi[a], Anna Barbui[b], Andrea Evangelista[c], Anna Morgando[a], Roberto Serra[b], Giovannino Ciccone[c], Alfredo Marzano[a], Mario Rizzetto[a]

[a] Division of Gastroenterology and Hepatology, AOU Città della Salute e della Scienza di Torino, University of Turin, Italy
[b] Laboratory of Microbiology, Department of Clinical Pathology, AOU Città della Salute e della Scienza di Torino, University of Turin, Italy
[c] Unit of Cancer Epidemiology, CPO Piemonte, AOU Città della Salute e della Scienza di Torino, University of Turin, Italy

ARTICLE INFO

Article history:
Received 11 November 2014
Accepted 16 April 2015
Available online 24 April 2015

Keywords:
Adrenal insufficiency
Ascites
Bacterial DNA
Cirrhosis

ABSTRACT

Background: There are few data on clinical relevance of adrenal dysfunction and its relationship with occult microbial DNA in noninfected haemodynamically stable cirrhotic patients with ascites.
Aims: The aim of this study was to evaluate prognostic role of adrenal dysfunction, microbial DNA, and their relationship.
Methods: Adrenal function was assessed in 93 consecutive patients following a corticotropin stimulation test. Adrenal dysfunction was defined as: basal cortisol <10 μg/dl, delta cortisol <9 μg/dl, or peak cortisol <18 μg/dl. Microbial DNA was assessed in blood and ascites of 54 consecutive patients. Patients were followed up until liver transplantation or death.
Results: Adrenal dysfunction was not significantly associated with mortality, while the risk of death rose significantly with an increase in basal cortisol values (HR 1.13 per 1-μl/dl increase; 95% CI 1.01–1.26). Microbial DNA was independently associated with reduced survival (HR 8.05, 95% CI 1.57–41.2). In microbial DNA-positive patients a significant correlation was found between Model for End-Stage Liver Disease (MELD) score and basal cortisol values (Pearson's $r = 0.5107$; $p = 0.018$).
Conclusions: Microbial DNA and MELD score, but not adrenal function, were the best independent predictors of mortality in noninfected cirrhotic patients with ascites. High serum cortisol levels may be a systemic reaction to microbial translocation, increasing in parallel with deterioration of liver function.

1. Introduction

Adrenal dysfunction (AD) is defined as inadequate cellular corticosteroid activity for the severity of the patient's illness [1]. It is common in patients with septic shock, and it is associated with circulatory collapse and hyporesponsiveness to vasopressors [2,3]. Intravenous administration of hydrocortisone reduces the time of shock reversal and the need for vasopressors; however, data on mortality are contradictory [4–6].

AD has also been investigated in patients with liver disease, adopting the same definitions used in the general population of critically ill patients [1–3]. Most studies were performed in patients with acute liver failure or septic shock: in this context, AD is common and it is associated with higher mortality rates. Hydrocortisone infusion showed encouraging, although non-univocal, results [7–11].

In patients with advanced cirrhosis without sepsis or other acute distress, data are limited [12–16]. A very recent study confirmed the high prevalence and the relevance of AD in noncritically ill cirrhotic patients, as patients with AD are at higher risk of circulatory and renal dysfunction, sepsis, and death [12].

In cirrhosis, AD was related to deficient hepatic synthesis of cortisol precursors (high-density lipoprotein (HDL) cholesterol and A1 apolipoprotein) due to liver insufficiency together with high

* Corresponding author at: Division of Gastroenterology and Hepatology, AOU Città della Salute e della Scienza di Torino, University of Turin, Corso Bramante 88, Turin 10126, Italy. Tel.: +39 011 6335561/5569/6397; fax: +39 011 6335714/5927.
E-mail address: risso.ale@gmail.com (A. Risso).

http://dx.doi.org/10.1016/j.dld.2015.04.010
1590-8658/

levels of circulating inflammatory cytokines, secondary to bacterial translocation from the gut [1,17]. This relationship was further investigated in recent years with the introduction of bacterial DNA measurement in blood and ascites to assess, at a molecular level, the presence of microorganisms in apparently noninfected cirrhotic patients [18–22], and with the demonstration that bacterial DNA is an independent predictor of poor survival in these patients [19]. Similar findings were also suggested for fungal DNA [23].

This prospective study evaluated the prognostic value of adrenal function, the presence and the clinical relevance of microbial DNA, and their relationship in noninfected cirrhotic patients with ascites.

2. Methods

All consecutive patients with cirrhosis and ascites admitted to the Gastroenterology and Hepatology Unit (19 beds) of our academic hospital between August 2008 and April 2011 were considered for inclusion. The diagnosis of cirrhosis was based on clinical, laboratory, and ultrasonographic findings. The inclusion criteria were as follows: cirrhosis with noninfected ascites (polymorphonuclear leukocytes <250/μl and negative ascitic fluid cultures), age between 18 and 75 years, and ability to provide written informed consent. The exclusion criteria were: shock, infection, or signs of bleeding within a week prior to inclusion; treatment with steroids or vasoactive drugs; multifocal hepatocellular carcinoma; alcoholic hepatitis; intrinsic renal failure; and heart or respiratory failure.

Patients underwent a clinical workup; blood, urine, and ascites were collected from all patients. Hepatic and renal function, activation of neurohormonal endogenous systems, and the levels of steroid synthesis precursors were determined.

The presence of an underlying infection was ruled out by white blood cell count; paracentesis with polymorphonuclear leucocyte count; ascites, blood, and urine cultures; chest X-ray; and ultrasonography.

Patients were followed up until liver transplantation (LT) or death. All procedures were in accordance with the ethical standards of committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Written informed consent was obtained from all patients.

2.1. Assessment of adrenal function

Patients underwent a short corticotropin stimulation test (SST) [24,25].

The serum total cortisol level was assessed at 8.00 a.m.; then, 250 μg of synthetic adrenocorticotrophic hormone (ACTH) (tetracosactide: Synachten, NOVARTIS FARMA S.p.A., Naples, Italy) was intravenously administered, and the serum total cortisol level was redetermined 60 min after the infusion.

2.2. Definitions of AD

Adrenal function was determined from the values of serum total cortisol before and 60 min after the administration of synthetic ACTH, using the following three standard criteria of AD: (a) "Basal cortisol": serum total cortisol before SST <10 μg/dl [1,2]; (b) "Delta cortisol": difference between serum total cortisol after and before SST <9 μg/dl [7,9]; (c) "Peak cortisol": serum total cortisol after SST <18 μg/dl [8,10].

2.3. Methods of measurement

The normal values of serum total cortisol in our laboratory are 4–20 μg/dl. The serum total cortisol level was measured using electrochemiluminescence-based methods on the Cobas e601 (Roche Diagnostics, Meylan, France). The intra-assay coefficient of variation (CV) is 1.4% in the cortisol range of 4–20 μg/dl.

The plasma renin activity (PRA) and plasma aldosterone levels were measured by radioimmunoassay. The normal values for PRA and plasma aldosterone in our laboratory are 0.1–4 ng/ml/h and 12–150 pg/ml, respectively.

The C-reactive protein (CRP) normal range in our laboratory is 0–3 mg/l.

2.4. Bacterial and fungal DNA

Blood and ascitic fluid were collected from all patients for bacterial and fungal cultures by inoculation of 10 ml of the sample in aerobe and anaerobe culture medium of the BactAlert automatic system (Biomerieux, Lyon, France) for 5 days. Determination of bacterial and fungal DNA in serum, blood, and ascites was also performed as soon as microbial DNA assay became available in our hospital (October 2009). Microbial DNA was extracted from 200 μl of serum or from the pellet of 5 ml of ascitic fluid using the EZ1 automatic system (Qiagen, Hilden, Germany), and from 1 ml of whole blood with the silica column manual system (Molzym, Bremen, Germany). The serum and ascitic fluid samples were eluted in 50 μl of elution buffer, while the whole blood samples were eluted in 100 μl of elution buffer, and 10 μl of the extracted DNA was examined for bacterial and fungal DNA by polymerase chain reaction (PCR).

Bacterial DNA was detected by amplifying a 500-bp region at the 5′ end of 16 S ribosomal RNA (rRNA) gene using a MicroSeq500 16sDNA Bacterial Identification PCR kit, and the sequencing reactions were performed with the MicroSeq500 16sDNA Bacterial Identification Sequencing kit (Applied Biosystems). In brief, 10 μl of the extracted DNA was mixed with the reaction mixture provided in the kit and amplified. The cycling conditions used were as follows: 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s. To determine the presence of a PCR product, 5 μl of the PCR reaction was run in a 2.0% agarose gel. The size of the PCR product was about 500 bp (460–560 bp, depending on the bacterial species).

Fungal DNA was detected by amplifying the genomic region internal transcribed spacer 2 (ITS2) rDNA (ITS1: 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS4: 5′-TCC TCC GCT TAT TGA TAT GC-3′). In brief, 10 μl of the extracted DNA was mixed with a reaction PCR mixture containing the enzyme and buffers (Master Mix PCR Qiagen) and 25 pmol of each primer; the cycling conditions used were as follows: 40 cycles of 95 °C for 60 s, 42 °C for 60 s, and 72 °C for 60 s. To determine the presence of a PCR product, 5 μl of the PCR reaction was run in a 2.0% agarose gel. The size of the PCR product was about 700 bp, depending on the species.

The absence of PCR inhibitors was confirmed by β-globin gene PCR detection in all extracted DNA samples. DNA extraction and detection of bacterial and fungal DNA were performed using 10 samples of serum and 10 samples of whole blood from blood donors to control the sterility of sampling, DNA extraction, and DNA amplification protocols.

The PCR products were identified by sequencing the amplicons obtained.

The nucleotide sequences of the PCR products were determined using an ABI PRISM Dye Terminator Cycle Sequencing v2.0 Ready Reaction Kit (Perkin Elmer, Foster City, CA, USA) and an ABI PRIMS 377 automated sequencer. The sequences were identified using BLAST at the National Center for Biotechnology Information web site (www.ncbi.nlm.nhi.gov).

2.5. Statistical analysis

The cumulative incidence of death (CID) was determined from the date of SST to the date of death or of LT. Patients who