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Bacterial DNA in non-leukocytic ascites, identification of risk factors

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Background: As culture-dependent bacterial identification methods are still limited to capture colonisation or infection of ascites fluid we established a culture-independent PCR-based method for the detection, quantification and differentiation of bacterial DNA (bactDNA). This report aims to characterize risk factors for bactDNA identification in non-leukocytic ascites fluid (nlAF) and for progression to SBP.

Methods: 299 nlAF samples of 142 patients were collected between 02/2011 and 12/2012. BactDNA was detected using real-time PCR with primers targeting the 16S-rRNA-gene and differentiated by direct sequencing. Genetic polymorphisms (SNP) of receptors recognising bacterial components such as TLR (subtypes 1,2,4,6), CD14, NOD2 and MBL2 were identified by detecting and amplifying corresponding genes. Patients´ characteristics such as liver function (bilirubin, PT, albumin, creatinine, sodium), inflammatory values (CRP-level) and underlying disease were correlated with PCR-results.

Quantification of bacterial DNA in non-leukocytic ascites fluid

	TLR1 rs4833095 Genotyp		
	TT (n=34)	CT (n=13)	CC (n=4)
bactDNA copies/ml	2541,3571 ± 1,4817,4458*	1620 ± 2978	546 ± 489
	TLR1 rs5743618 Genotyp		
	GG (n=33)	GT (n=14)	TT (n=4)
bactDNA copies/ml	2618,3660 ± 1,5040,2764**	1550 ± 2870	546 ± 489

Table 1: Quantification of bacterial DNA in non-leukocytic but bactDNA positive ascites samples (indexparacentesis), mean ± standard deviation (copies/ml) determined by Mann-Whitney-U-test
* TT vs. CT/CC; p=0,016
** GG vs. GT/TT; p=0,021

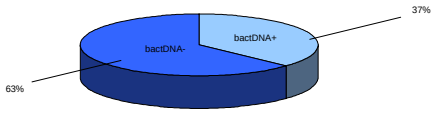

Figure 1: Detection of bactDNA in non-leukocytic ascites fluid (indexparacentesis). 37% samples were tested positive for bacterial DNA.

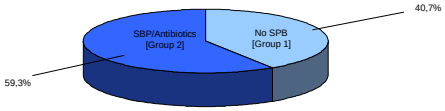

Figure 2: Spontaneous resolution (in 40,7 % of all cases) or progression to SBP or antibiotic therapy (in 59,3% of all cases) after bacterial DNA detection in non-leukocytic ascites.

Results

BactDNA could be detected in 37 % of nlAF of indexparacentesis [fig. 1]. The risk for bactDNA in indexparacentesis was associated with genetic polymorphism of TLR2 [rs5743708 G/A, fig. 3] and TLR6 [rs5743810 C/T, fig. 4]. The amount of bactDNA (copies/ml) was dependent on genetic variants of TLR1 [table 1] (rs5743618, G/T; rs4833095 T/C). Polymorphisms of CD14, NOD2 as well as MBL2 showed no association with bactDNA in ascites samples. After detection of bactDNA in nfAF 40,7% did not develop SBP (group 1) whereas 59% proceeded to SBP or received antibiotics (group 2) [fig. 2]. Group2 had significantly decreased prothrombin time and increased CRP levels [fig. 5] Group comparison did not reveal an association with genetic polymorphisms. Etiology of cirrhosis did not influence the detection rate of bactDNA.

Conclusion

These results emphasise that the susceptibility to bacterial colonisation or ascites infection in patients with cirrhosis might be genetically determined. However, further risk factors and mechanisms favouring the progression from colonisation to overt SBP still have to be clarified.

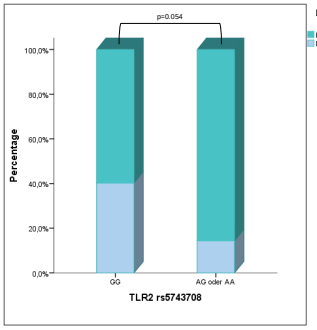

Figure 3: Detection rate of bacterial DNA in non-leukocytic ascites (indexparacentesis) depending on TLR2 rs5743708 mutation (protective allel A, OR 0,241, p=0,054), (GG n=122, AG/AA n=14)

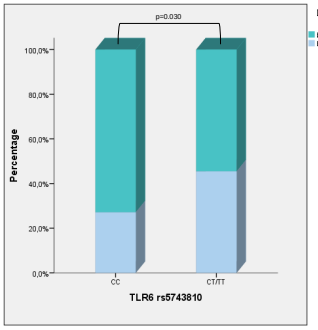

Figure 4: Detection rate of bacterial DNA in non-leukocytic ascites (indexparacentesis) depending on TLR6 rs5743810 mutation (risk allel T, OR 2,241, p=0,030), (CC n=59, CT/TT n=77)

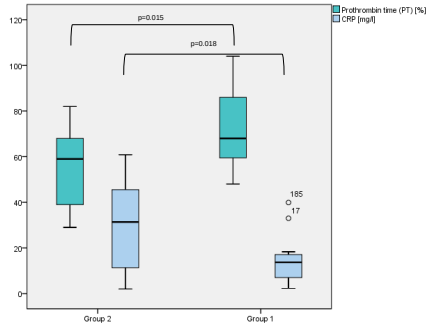

Figure 5: Differences of laboratory values between patients proceeding to SBP or antibiotic therapy (group 2) and spontaneous resolution of bacterial DNA in ascites fluid (group 1). Patients in group 1 had significantly increased PT (mean 73,3 ± 17,3 % vs. 55,9 ± 16 %, p=0,015) and decreased CRP-levels (mean 15,9 ± 11,9 mg/l vs. 33,4 ± 23,8 mg/l, p=0,018).

References: 1: Krohn S et al. 2014; 2: Nischalke et al. 2011; 3: Appenroth B et al. 2010; 4: Jalan R et al. 2014

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