Plasma proteomic analysis identified proteins associated with faulty neutrophils functionality in decompensated cirrhosis patients with sepsis

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SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY TABLE:

Supplementary Table S1: Lowest detection limit of cytokines, chemokines, and growth factors in cytokine bead array assay.

S. No.	Name	Lowest detection limit (pg/ml)
1	IL-6	10
2	IL-2	5.71
3	IL-4	9.99
4	IL-10	1.70
5	TNF-α	8.59
6	IFN- γ	15
7	GMCSF	15
8	MIF	0.21
9	IL-8	2.76
10	MCP-1	3.93
11	IP-10	2.03
12	GCSF	13
13	IL-1β	1.99
14	IL-18	17
15	TGF-b1	3
16	MIP-1A	2.04
17	MIP-1B	5.64
18	MIP-3A	4.44
19	ITAC	3.74
20	FRACTALKINE	1.25
21	ENA78	6.59
22	MCSF	14
23	LEPTIN	16
24	VEGF-A	5.37
25	MMP12	5.76
26	E SELECTIN	206
27	Angiopoietin-1	32
28	Eotaxin	0.62
29	HGF	6.49
30	IL-1RA	43
31	IL-12p40	1.35
32	IL-17A	2.27
33	IL-27	17
34	IL-33	23
35	MMP-1	8.52
36	MMP-13	7.96
37	MMP-7	11
38	MMP-8	36
39	P-SELECTIN	1348
40	TPO	94
41	TREM-1	689

Supplementary Table S2: Correlation of upregulated and downregulated proteins with the neutrophils, its oxidative burst activity, MELD, MELD Na and Bilirubin. R-value and p-value given in each cell. Cell containing no value shows no significant correlation.

	DNAJC13	GPI	GASTP1	PNP	ANPEP	COTL1	PGMA1	APOA1	PROS1	SERPINA3	MPO	MMRN1
NEUTROPHILS	0.945 0.002	0.994 <0.0001	0.879 0.021	0.885 0.019	0.915 0.010	0.977 <0.0001	0.905 0.013	-0.896 0.015	0.970 0.001	0.927 0.007	0.903 0.013	0.882 0.020
$CD11b^{+}$ NEUTRO	0.835 0.038	-	-	-	0.840 0.036	0.869 0.024	0.856 0.039	-	0.933 0.006	0.864 0.026	0.879 0.020	-
OXIDATIVE BURST	0.746 0.050	-	-	-	-	0.824 0.043	0.899 0.014	-	0.944 0.004	0.884 0.019	-	-
MELD	0.916 0.010	0.967 0.001	0.816 0.047	0.794 0.050	0.920 0.001	0.906 0.012	0.809 0.050	-0.836 0.037	0.906 0.013	0.828 0.041	0.957 0.002	-
MELD Na	0.953 0.003	0.980 <0.0001	0.881 0.020	0.870 0.024	0.934 0.006	0.958 0.002	0.855 0.029	-0.881 0.020	0.936 0.006	0.880 0.020	0.938 0.005	0.838 0.037
Bilirubin	0.976 <0.0001	0.972 0.001	0.933 0.006	0.908 0.012	0.966 0.001	0.987 <0.0001	0.834 0.039	-0.863 0.026	0.921 0.009	0.895 0.015	0.915 0.010	0.868 0.002

Supplementary Table S3: Correlation of neutrophils, its phagocytic and oxidative burst activity with the cytokines, chemokine and growth factors. R-value and p-value given in each cell. Cell containing no value shows no significant correlation.

	IL-10	TPO	MIP-1α	IL-33	MCSF	MCP1	HGF	ENA78	MIP3α	MMP1	PSELECTIN	TREM1	IL-18	IL-4	IFN-γ	TNF-α	MMP7	VEGF-A	IL-12p40	IL-1β	TGF-β1	MIP-1β	ESELECTIN	MIF	IL-27	EOTAXIN	IL-17A	IL-2	IP-10
NEUTROPHILS	.	.	.	0.746 0.027	.	0.800 0.050	0.867 0.025	0.808 0.050	0.902 0.014	0.875 0.022	0.878 0.021	0.957 0.003	0.980 <0.0001	0.958 0.002	0.992 <0.0001	0.966 0.002	0.959 0.002	0.972 0.001	0.967 0.001	0.915 0.010	0.956 0.003	0.834 0.039	.	0.891 0.017	0.932 0.006	0.936 0.005	0.939 0.005	0.969 0.001	0.973 0.001
$CD11b^+$ NEUTRO	.	.	.	0.863 0.027	.	.	.	.	.	.	.	0.880 0.020	0.963 0.015	0.855 0.029	0.914 0.011	0.851 0.032	0.873 0.023	0.859 0.028	0.851 0.031	0.833 0.039	0.958 0.026	0.857 0.029	.	0.844 0.034	0.947 0.004	0.939 0.005	0.879 0.021	0.932 0.006	0.960 0.002
$CD11b^+CXCR1^+$	0.865 0.026	.	.		0.862 0.027	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PHAGOCYTIC ACTIVITY	.	-0.915 0.010	-0.913 0.010	-0.862 0.027	.	.	.	.	.	.	.	.	.	.	.	.	-0.816 0.047	.	.	.	.	-0.813 0.048	-0.832 0.039	-0.831 0.040	-0.877 0.022	-0.873 0.023	-0.874 0.023	-0.823 0.044	.
OXIDATIVE BURST	.	.	.	.	.	.	.	.	.	.	.	.	0.903 0.042	0.849 0.032	0.883 0.019	0.821 0.045	0.811 0.050	0.796 0.050	.	0.863 0.027	0.977 0.000	.	.	.	0.934 0.006	0.893 0.016	0.868 0.025	0.924 0.008	0.959 0.002

Supplementary Table S4: Correlation of upregulated and downregulated proteins with the cytokines, chemokine and growth factors. R-value and p-value given in each cell. Cell containing no value shows no significant correlation.

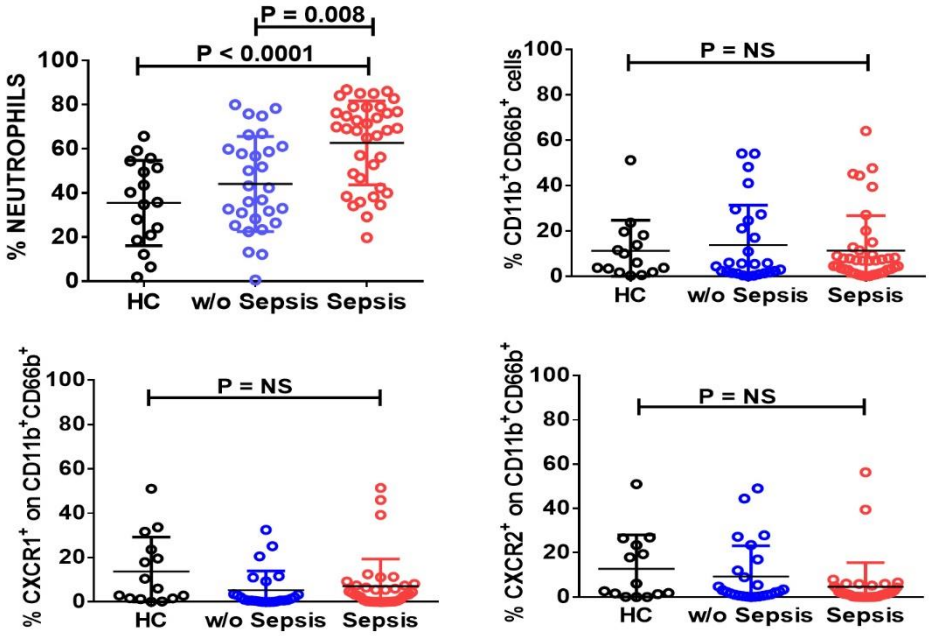
	MIP-1α	ANGIOPOIENTIN	MCSF	IL-8	MMP8	MCP1	HGF	ENA78	MIP3a	MMP1	PSELECTIN	TREM1	IL-18	IL-4	IFN-γ	TNF-α	MMP7	VEGF-A	IL-12p40	IL-1β	TGF-β1	MIP-1β	ESELECTIN	MIF	IL-27	EOTAXIN	IL-17A	IL-2	IP-10
DNAJC13	-	-	-	0.837 0.037	-	0.943 0.005	0.973 0.001	0.899 0.014	0.968 0.001	0.934 0.006	0.961 0.002	0.945 0.004	0.926 0.008	0.906 0.013	0.944 0.004	0.947 0.004	0.981 <0.0001	0.985 <0.0001	0.986 <0.0001	0.807 0.050	0.823 0.044	0.858 0.028	0.871 0.023	0.947 0.004	0.854 0.030	0.900 0.014	0.899 0.015	0.900 0.014	0.874 0.022
GPI	-	-	-	-	-	-	0.835 0.038	-	0.874 0.023	0.848 0.033	0.834 0.038	0.960 0.002	0.987 <0.0001	0.943 0.005	0.984 <0.0001	0.945 0.004	0.923 0.008	0.950 0.003	0.939 0.005	0.916 0.010	0.959 0.002	0.804 0.050	-	0.850 0.032	0.912 0.011	0.910 0.011	0.905 0.013	0.950 0.004	0.965 0.002
GSTP1	-	-	0.839 0.036	0.924 0.008	0.885 0.019	0.0049	0.983 <0.0001	0.959 0.002	0.985 <0.0001	0.966 0.001	0.992 <0.0001	0.895 0.016	0.839 0.036	0.885 0.019	0.899 0.014	0.931 0.007	0.947 0.004	0.964 0.002	0.968 0.001	-	-	-	0.802 0.050	0.893 0.016	-	0.813 0.049	0.857 0.029	0.838 0.037	-
PNP	-	0.824 0.043	-	0.906 0.013	0.860 0.028	0.884 0.019	0.925 0.008	0.985 <0.0001	0.965 0.002	0.957 0.002	0.994 0.000	0.849 0.032	0.809 0.050	0.932 0.007	0.913 0.010	0.959 0.002	0.953 0.003	0.956 0.003	0.971 0.001	0.845 0.034	-	-	-	0.863 0.026	0.806 0.050	0.826 0.042	0.916 0.010	0.888 0.018	0.842 0.035
ANPEP	-	-	0.937 0.005	-	-	0.886 0.018	0.952 0.003	-	0.917 0.009	0.875 0.022	0.861 0.027	0.978 <0.0001	0.952 0.003	0.819 0.046	0.901 0.014	0.866 0.025	0.895 0.015	0.937 0.006	0.918 0.009	-	-	0.835 0.038	0.824 0.043	0.890 0.017	-	0.830 0.040	-	0.805 0.050	0.803 0.050
COTL1	-	-	-	-	-	0.821 0.045	0.921 0.009	0.899 0.015	0.967 0.001	0.956 0.003	0.945 0.004	0.963 0.002	0.942 0.005	0.978 <0.0001	0.993 <0.0001	0.992 <0.0001	0.954 0.003	0.994 <0.0001	0.990 <0.0001	0.930 0.007	0.904 0.013	-	-	0.856 0.029	0.858 0.028	0.868 0.024	0.918 0.009	0.935 0.006	0.925 0.008
GCA	-	-	0.797 0.050	0.977 <0.0001	0.938 0.005	0.946 0.004	0.954 0.003	0.960 0.002	0.945 0.004	0.926 0.008	0.975 <0.0001	0.799 0.050		0.808 0.050	0.810 0.050	0.864 0.026	0.901 0.014	0.900 0.014	0.913 0.010	-	-	-	-	0.861 0.028	-	-	0.803 0.050	-	-
PGAM1	-	-	-	-	-	-	-	-	-	-	-	-	0.819 0.045	0.960 0.002	0.923 0.008	0.926 0.008	0.849 0.032	0.856 0.029	0.866 0.026	0.979 0.000	0.967 0.001	-	-	-	0.889 0.017	0.829 0.041	0.937 0.006	0.952 0.003	0.955 0.003
APOA1	-	-	-	-	-	-0.831 0.040	-0.814 0.048	-0.903 0.013	-0.863 0.026	-0.841 0.036	-0.917 0.009	-	-0.807 0.050	-0.924 0.008	-0.900 0.014	-0.934 0.006	-0.963 0.002	-0.911 0.011	-0.937 0.006	-0.849 0.032	-0.872 0.023	-	-0.813 0.049	-0.891 0.017	-0.921 0.009	-0.917 0.009	-0.985 0.000	-0.955 0.003	-0.917 0.009
AHSG	-	0.875 0.022	-	-	-	-	-	0.841 0.035	-	-	0.799 0.050	-		0.898 0.015	0.810 0.050	0.868 0.025	-	-	0.805 0.050	0.896 0.015	0.831 0.040	-	-	-	-	-	0.903 0.013	0.870 0.024	0.839 0.036
PROS1	-	-	-	-	-	-	-	-	0.837 0.037	0.829 0.041	0.819 0.046	0.891 0.017	0.924 0.008	0.974 0.001	0.975 0.000	0.954 0.003	0.897 0.015	0.919 0.009	0.918 0.009	0.975 0.009	0.989 <0.0001	-	-	-	0.916 0.010	0.882 0.019	0.936 0.005	0.970 0.001	0.982 <0.0001
SERPINA3	-	0.841 0.036	-	-	-	-	-		0.835 0.038	0.848 0.032	0.798 0.050	0.883 0.019	0.881 0.020	0.963 0.002	0.954 0.003	0.937 0.005	0.824 0.043	0.893 0.016	0.882 0.019	0.990 0.000	0.942 0.005	-	-	-	0.812 0.049	-	0.860 0.027	0.903 0.014	0.920 0.009
MPO	0.815 0.482	-	0.831 0.040	-	-	0.927 0.007	0.888 0.018	-	0.832 0.039	-	0.817 0.047	0.893 0.016	0.929 0.007	-	0.859 0.028	0.823 0.044	0.933 0.006	0.894 0.016	0.892 0.017	-	-	0.971 0.001	0.963 0.002	0.981 <0.0001	0.875 0.022	0.938 0.005	0.835 0.038	0.852 0.031	0.842 0.035
MMRN1	-	0.926 0.007	-	-	-	-	-	0.943 0.004	0.905 0.013	0.921 0.008	0.930 0.007	-	-	0.976 0.000	0.925 0.008	0.969 0.001	0.893 0.016	0.918 0.009	0.931 0.007	0.949 0.004	0.864 0.026	-	-	-	0.807 0.050	-	0.926 0.007	0.910 0.011	0.881 0.020

Supplementary Table S5: Sequence of primers used for RT-PCR

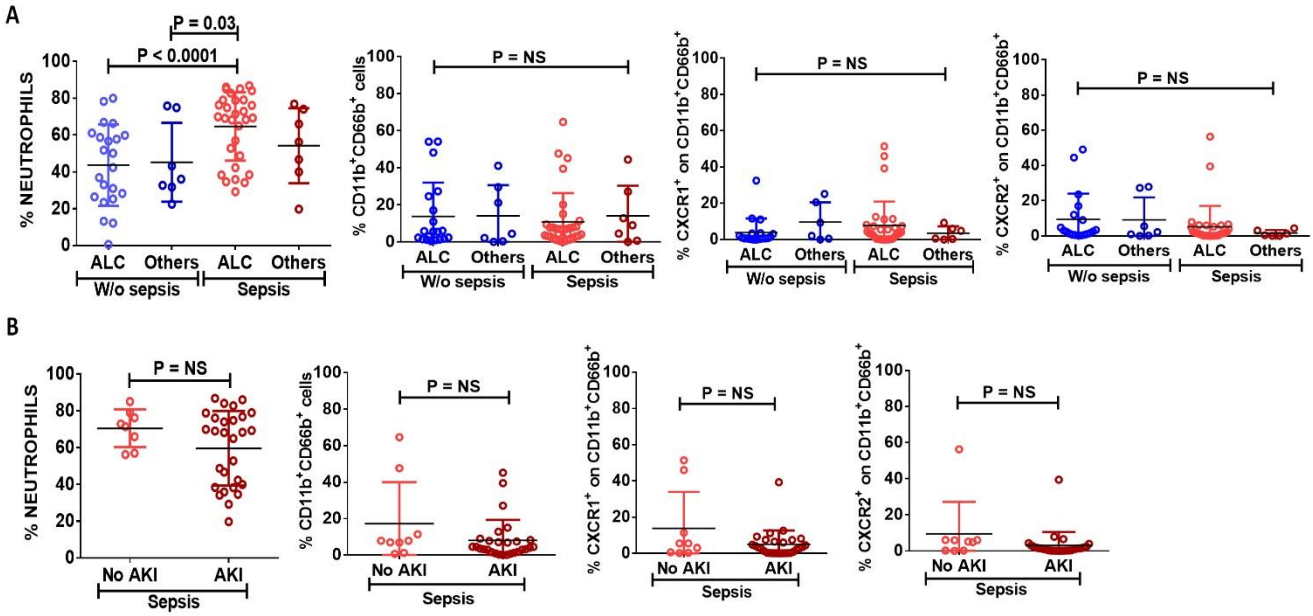
Name	Forward primer	Reverse primer
18s	AAGTACGCACGGCCGGTACA	AGCGCCCGTCGGCATGTATTA
DNAJC13	AGTGGCAAAGACTAGCTGGAA	TGTCCTCTTTTTGAGCAATGCC
TMSB4X	ACTGCCTTCCAAAGAAACGAT	AGGGGCAGCACAGTCATTTA
AHSG	CATGGCCCAGGGCTGATTTA	CTGCTGAGGCCACACCTTTA
HMGB1	ATA TGGCAAAAGCGGACAAG	AGGCCAGGATGTT CTCCTTT
HIF-1α	GTTTACTAAAGGACAAGTCACC	TTCTGTTTGTTGAAGGGAG
ATG5	TTGACGTTGGTAACTGACAAAGT	TGTGATGTTCCAAGGAAGAGC
ATG12	TAGAGCGAACACGAACCATCC	CACTGCCAAAACCATCATAGAGA

SUPPLEMENTARY FIGURES:

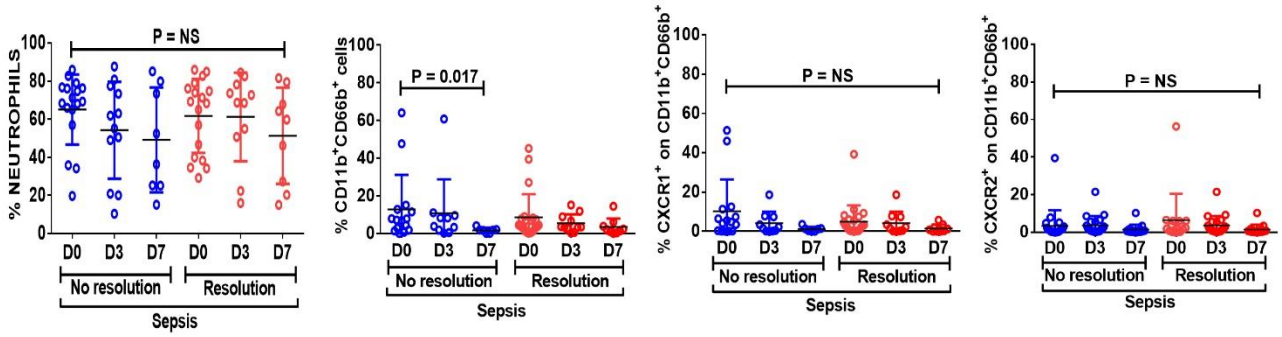
SUPPLEMENTARY FIGURE S1: Scatter dot plot shows the percent expression of Neutrophils, $CD11b^+CD66b^+$ cells, $CXCR1^+$ and $CXCR2^+$ on $CD11b^+CD66b^+$ neutrophils in sepsis and w/o sepsis at different time points i.e D0, D3 and D7.



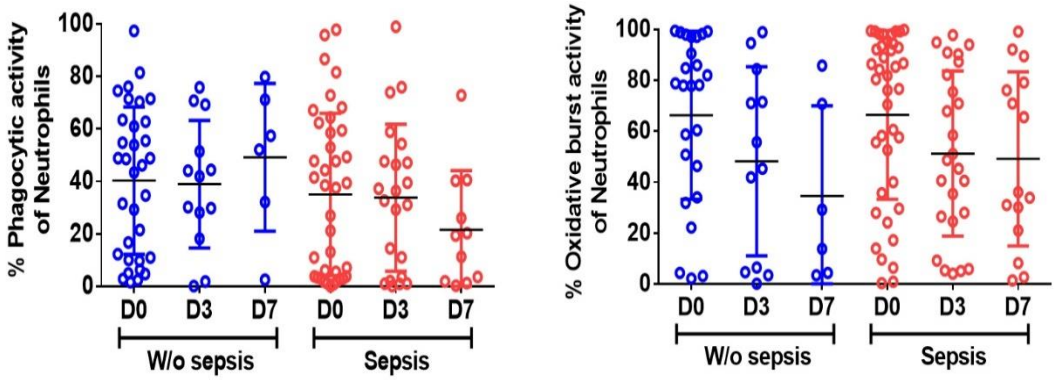
SUPPLEMENTARY FIGURE S2: Scatter dot plot shows the percent expression of Neutrophils, $CD11b^+CD66b^+$ cells, $CXCR1^+$ and $CXCR2^+$ on $CD11b^+CD66b^+$ neutrophils **A)** based on alcohol (ALC) and other etiologies (NASH and cryptogenic) in patients with or w/o sepsis; **B)** with or w/o AKI as a decompensating event in sepsis patients.



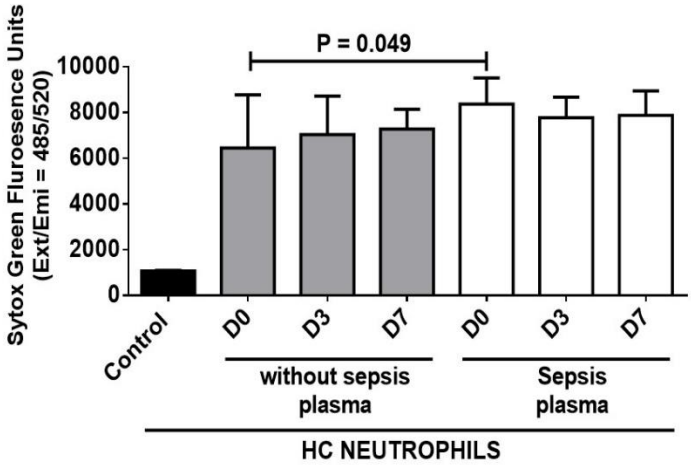
SUPPLEMENTARY FIGURE S3: Scatter dot plot shows the percent expression of Neutrophils, $CD11b^+CD66b^+$ cells, $CXCR1^+$ and $CXCR2^+$ on $CD11b^+CD66b^+$ neutrophils based on no resolution and resolution of sepsis upon similar treatment in decompensated cirrhosis with sepsis patients at D0, D3 and D7.



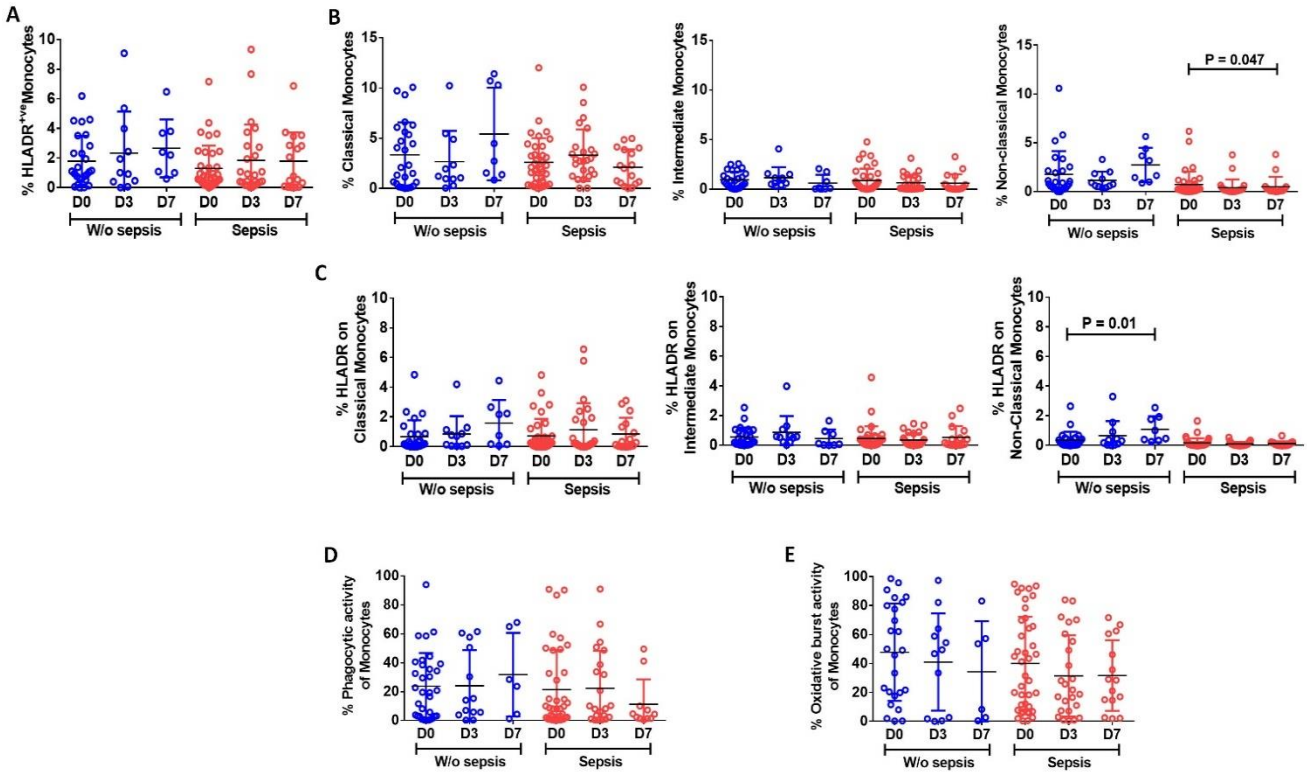
SUPPLEMENTARY FIGURE S4: Scatter dot plot shows the percent expression of phagocytic activity and oxidative burst activity (*E. coli*) of neutrophils in sepsis and w/o sepsis at D0, D3 and D7.



SUPPLEMENTARY FIGURE S5: Cell free DNA in sepsis and w/o sepsis at different time points i.e D0, D3 and D7.



SUPPLEMENTARY FIGURE S6: Percent expression of **A)** HLA-DR on monocytes **B)** classical, intermediate, and non-classical monocytes **C)** HLA-DR on classical, intermediate, and non-classical monocytes, **D)** phagocytic and **E)** oxidative burst activity (E. coli) of monocytes in sepsis and w/o sepsis at different time points i.e D0, D3 and D7.



SUPPLEMENTARY FIGURE S7: Percent expression of **A)** HLA-DR on monocytes **B)** classical, intermediate, and non-classical monocytes based on no resolution and resolution of sepsis upon similar treatment in decompensated cirrhosis with sepsis patients at D0, D3 and D7.