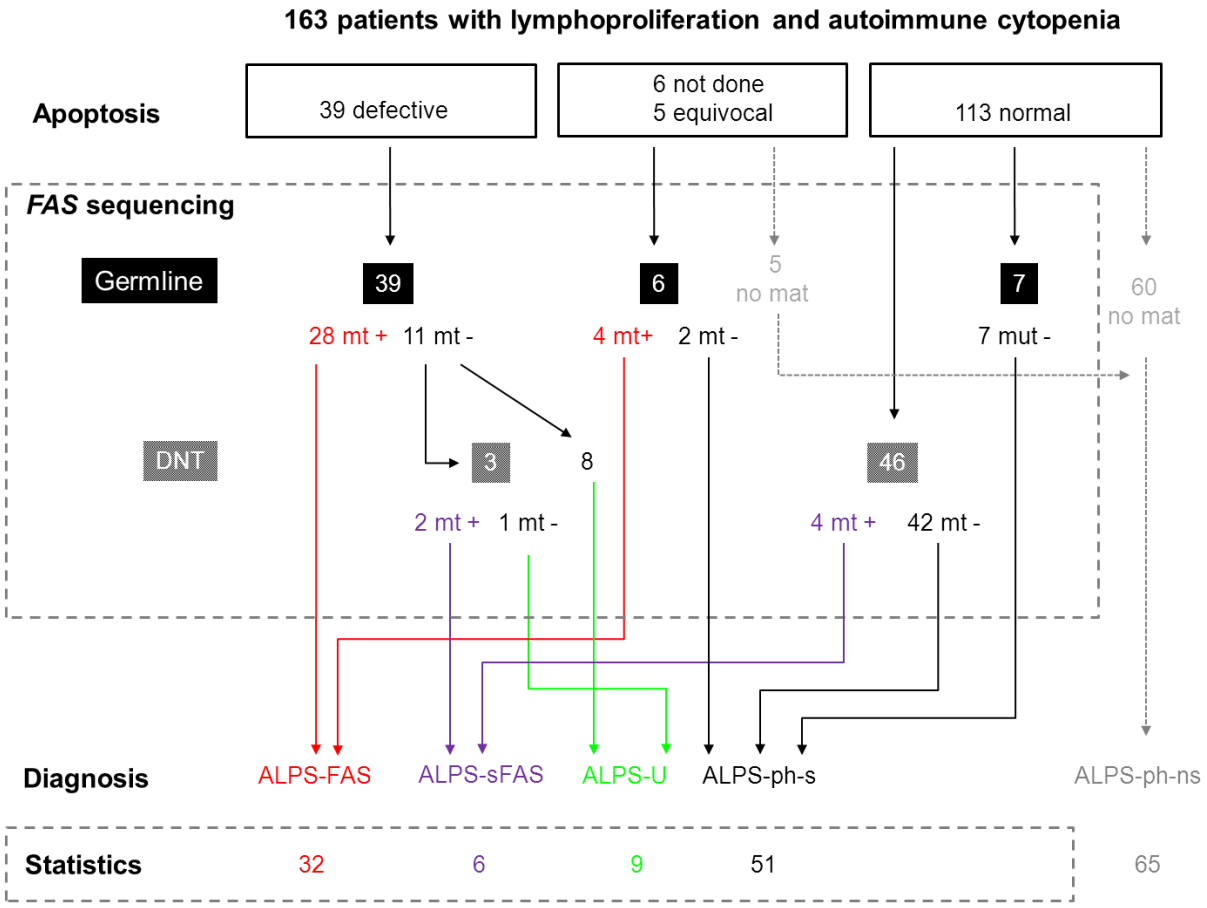
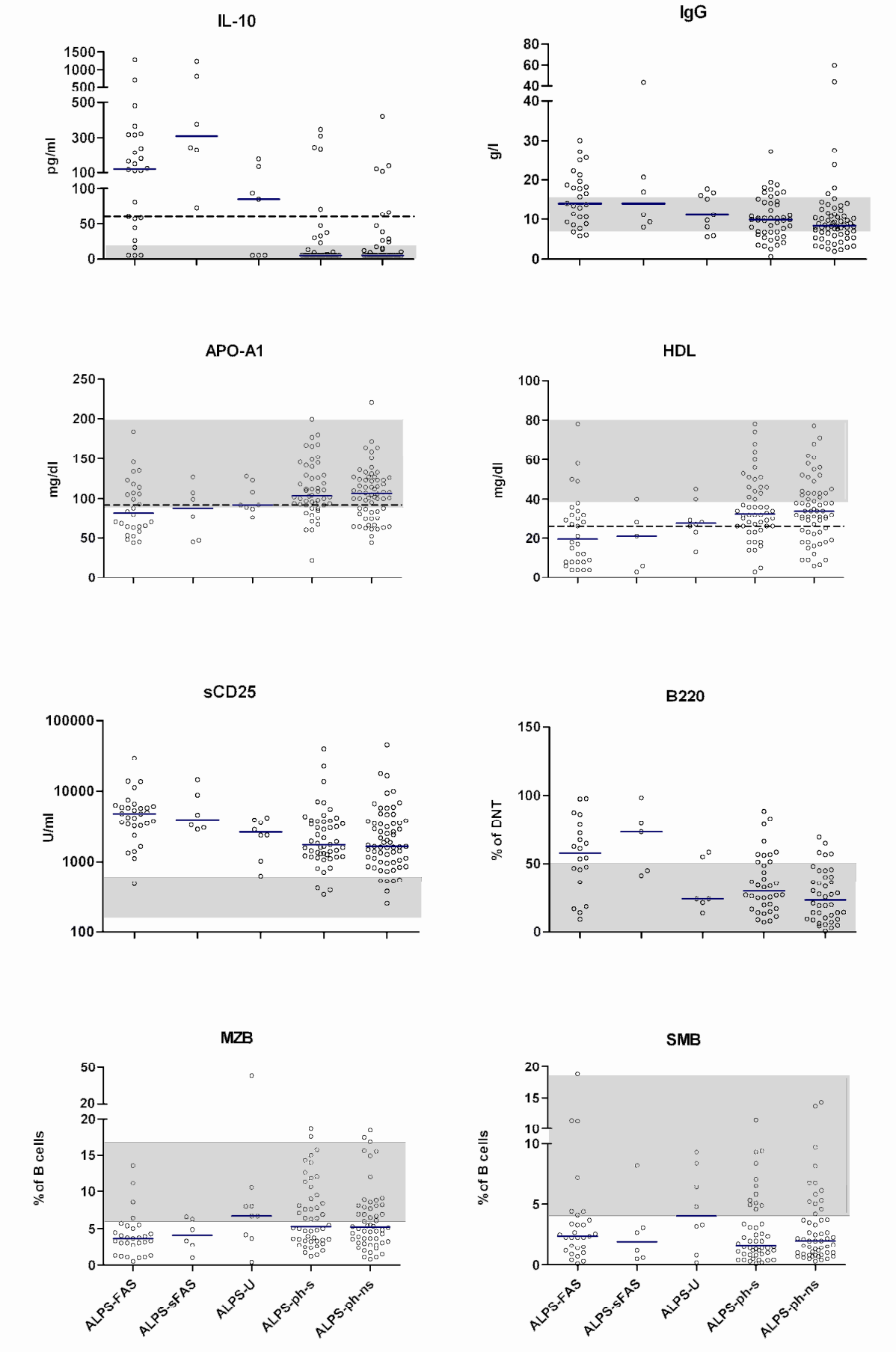
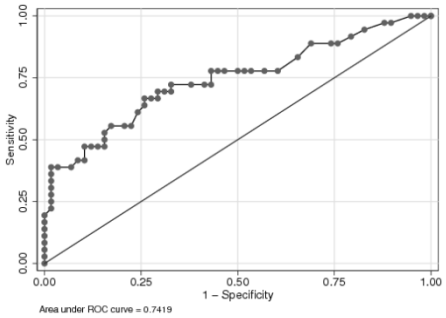


Supplementary Figure 1: Diagnostic flow and classification of study patients. “mt+”: *FAS* mutation detected, “mt-“: no mutation detected in *FAS, FASL, CASP8* and *CASP10*, “no mat”: no material for genetic analysis available.

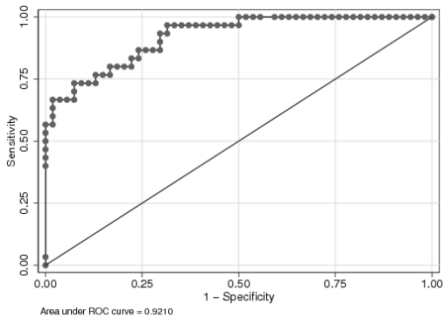

IL-10
pg/ml
1500
1000
500
500
300
100
100
50
0
IgG
g/l
80
60
40
40
30
20
10
0
APO-A1
mg/dl
250
200
150
100
50
0
HDL
mg/dl
100
80
60
40
20
0
sCD25
U/ml
100000
10000
1000
100
B220
% of DNT
150
100
50
0
MZB
% of B cells
50
20
20
15
10
5
0
SMB
% of B cells
20
15
10
10
5
0
ALPS-FAS
ALPS-sFAS
ALPS-U
ALPS-ph-s
ALPS-ph-ns

Supplementary Figure 2. Biomarker distribution in the analyzed patient groups for the remaining parameters. Shaded areas represent normal ranges. In the case of known age dependency (IgG, MZB, SMB), the variable normal ranges cannot be depicted for the whole cohort in one plot; the shown range represents normal values for the most prevalent age group in our cohort (10-18 years). The dashed lines in some plots indicate the cut-offs identified for selected parameters based on the prevalence of *FAS* mutations in our cohort as described in the methods section. P values for differences between ALP-FAS/sFAS and the other investigated groups were calculated using the Kruskal Wallis test and were found to be significant for IL-10, IgG, sCD25, B220 ($p<0.001$), HDL, APOA1, MZB ($p<0.05$) and not significant for SMB ($p=0.78$).

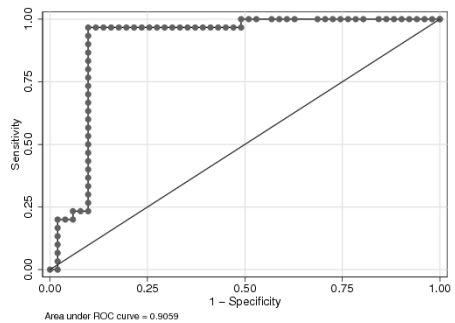
DNT



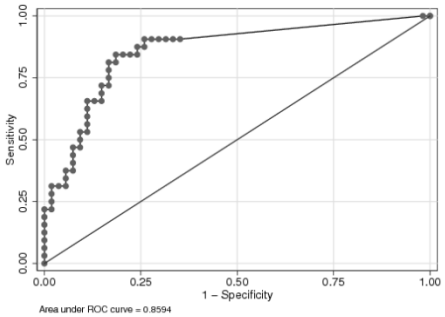
vitamin B12



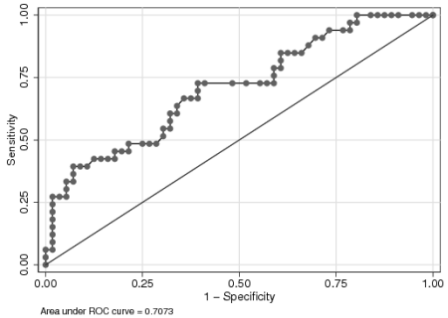
sFASL



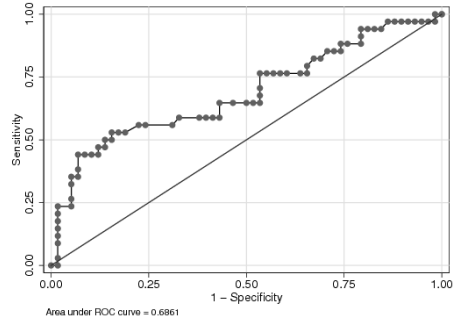
IL-10



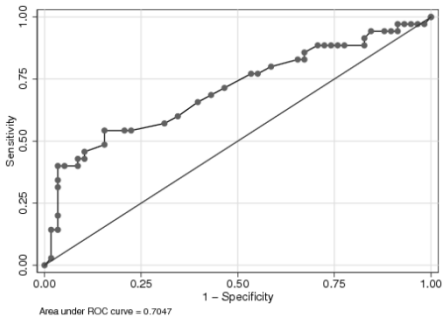
IgG



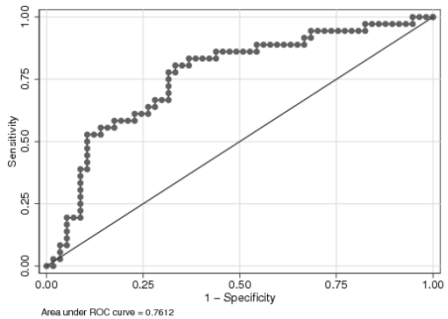
APO-A1



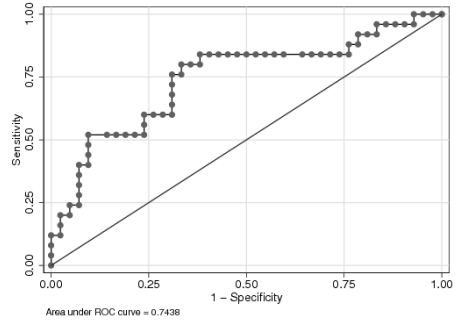
HDL



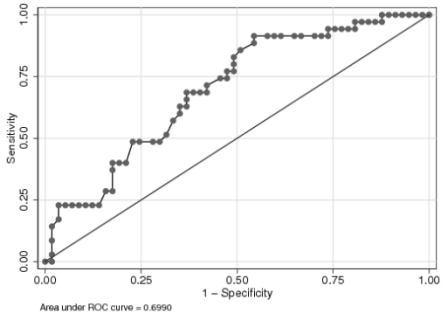
sCD25



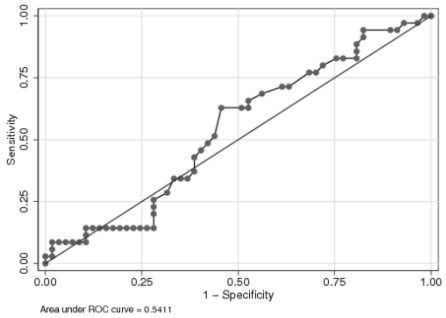
B220



MZB

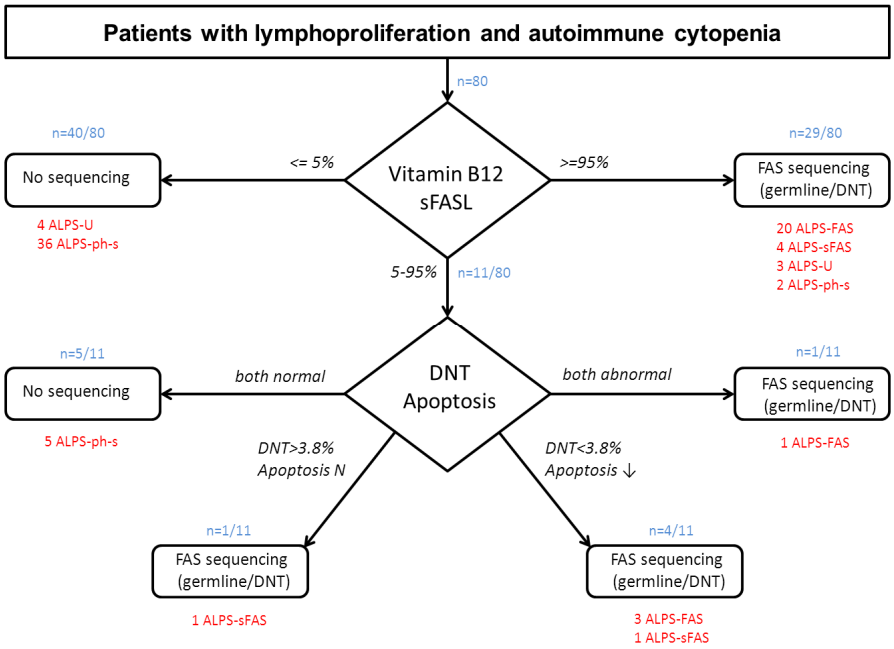


SMB

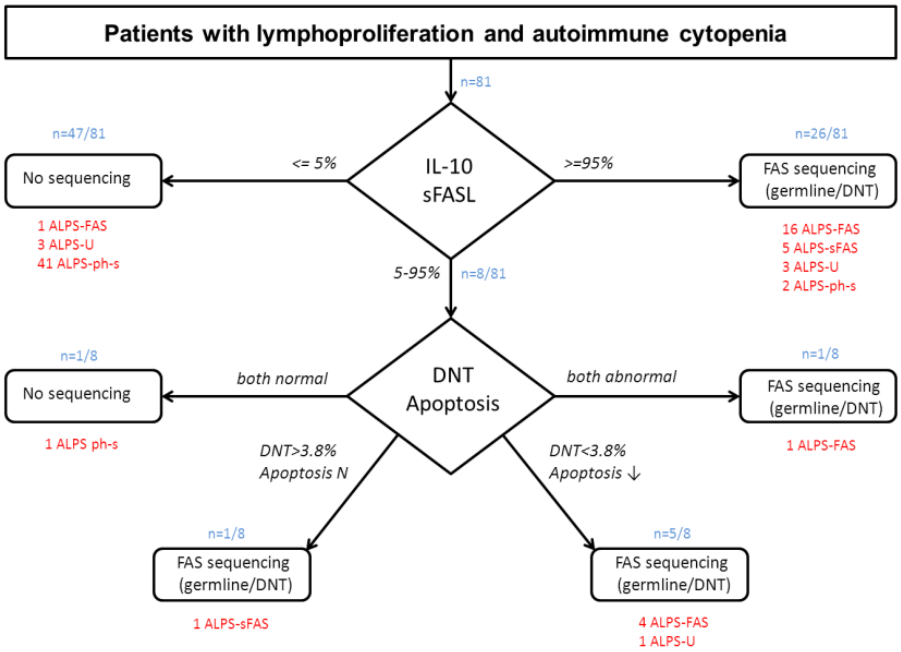


Supplementary Figure 3: ROC curves and corresponding AUC values of all measured parameters.

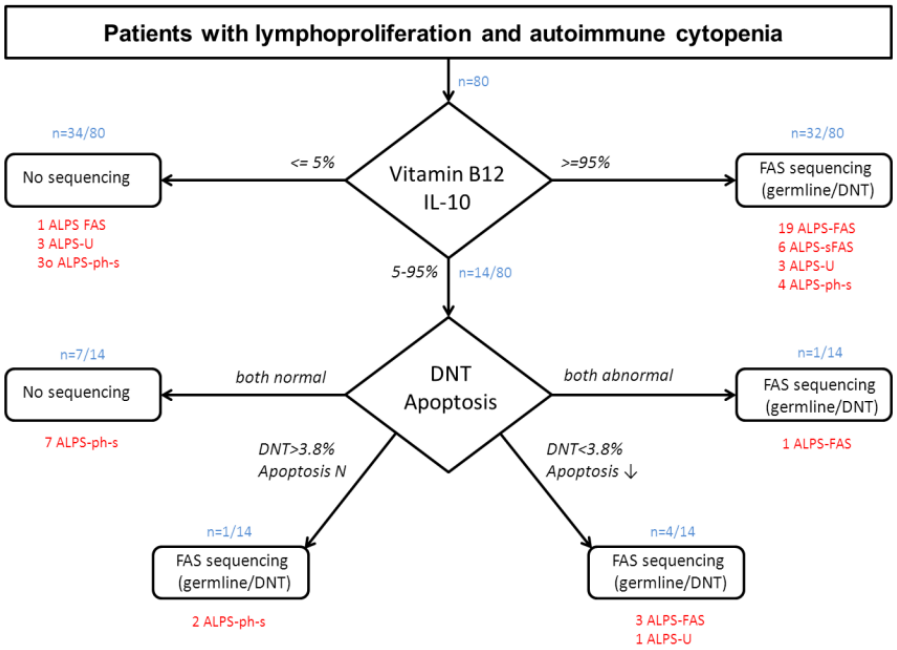
A


Patients with lymphoproliferation and autoimmune cytopenia
n=80
Vitamin B12
sFASL
<= 5%
>=95%
5-95%
n=11/80
n=40/80
No sequencing
4 ALPS-U
36 ALPS-ph-s
n=29/80
FAS sequencing
(germline/DNT)
20 ALPS-FAS
4 ALPS-sFAS
3 ALPS-U
2 ALPS-ph-s
n=5/11
No sequencing
5 ALPS-ph-s
both normal
DNT
Apoptosis
both abnormal
n=1/11
FAS sequencing
(germline/DNT)
1 ALPS-FAS
DNT>3.8%
Apoptosis N
DNT<3.8%
Apoptosis ↓
n=1/11
FAS sequencing
(germline/DNT)
1 ALPS-sFAS
n=4/11
FAS sequencing
(germline/DNT)
3 ALPS-FAS
1 ALPS-sFAS

B


Patients with lymphoproliferation and autoimmune cytopenia
n=81
IL-10
sFASL
<= 5%
>=95%
5-95%
n=8/81
n=47/81
No sequencing
1 ALPS-FAS
3 ALPS-U
41 ALPS-ph-s
n=26/81
FAS sequencing
(germline/DNT)
16 ALPS-FAS
5 ALPS-sFAS
3 ALPS-U
2 ALPS-ph-s
n=1/8
No sequencing
1 ALPS ph-s
both normal
DNT
Apoptosis
both abnormal
n=1/8
FAS sequencing
(germline/DNT)
1 ALPS-FAS
DNT>3.8%
Apoptosis N
DNT<3.8%
Apoptosis ↓
n=1/8
FAS sequencing
(germline/DNT)
1 ALPS-sFAS
n=5/8
FAS sequencing
(germline/DNT)
4 ALPS-FAS
1 ALPS-U

C


Patients with lymphoproliferation and autoimmune cytopenia
n=80
Vitamin B12
IL-10
<= 5%
>=95%
5-95%
n=14/80
n=34/80
No sequencing
1 ALPS FAS
3 ALPS-U
3o ALPS-ph-s
n=32/80
FAS sequencing
(germline/DNT)
19 ALPS-FAS
6 ALPS-sFAS
3 ALPS-U
4 ALPS-ph-s
n=7/14
No sequencing
7 ALPS-ph-s
both normal
DNT
Apoptosis
both abnormal
n=1/14
FAS sequencing
(germline/DNT)
1 ALPS-FAS
DNT>3.8%
Apoptosis N
DNT<3.8%
Apoptosis ↓
n=1/14
FAS sequencing
(germline/DNT)
2 ALPS-ph-s
n=4/14
FAS sequencing
(germline/DNT)
3 ALPS-FAS
1 ALPS-U

Supplementary Figure 4: Current diagnostic flow for patients with chronic splenomegaly or lymphoproliferation and autoimmunity at the diagnostic unit of the Centre of Chronic Immunodeficiency (CCI), Freiburg. The performance of the diagnostic flow with initial determination of (A) vitamin B12 and sFASL, (B) IL-10 and sFASL, or (C) vitamin B12 and IL-10 was retrospectively evaluated with subjects of this study cohort. The numbers of evaluated patients are depicted in blue; the final diagnoses are marked in red.

Country	Number of patients (%)
Germany	109 (67%)
United Kingdom	18 (11%)
Czech Republic	10 (6%)
Austria	9 (6%)
Ireland	5 (3%)
Finland	4 (3%)
Spain	3 (2%)
Portugal	3 (2%)
Slovakia	2 (1%)

Supplementary Table 1. Number of study patients per participating country.

Parameter	Number of patients analyzed	Number of patients with abnormal values (%)
IgG*	151	71 (47%)
Vitamin B12*	146	50 (34%)
sCD25 > 623 U/ml > 2400 U/ml	156	146 (94%) 80 (51%)
APO-A1 < 40 mg/dl	154	53 (34%)
HDL < 40 mg/dl	156	111 (71%)
IL-10 > 20 pg/ml	145	53 (37%)
sFASL > 200 pg/ml	140	58 (41%)
DNT > 1% > 2% > 4%	155	134 (86%) 91 (59%) 40 (26%)
B220 > 50%	107	32 (30%)
MZB*	146	88 (60%)
SMB*	145	109 (75%)
Apoptosis	157	39 (25%)

Supplementary Table 2. Data completeness and prevalence of abnormal values in our study cohort.

Number of patients analyzed for each parameter and number and percentage (in parenthesis) of patients with abnormal values in the evaluated parameter are shown.

* age related normal values.

Diagnosis	IgG	Vitamin B12	sCD25	APO-A1	HDL	IL-10	sFASL	DNT	B220	MZB	SMB	Apoptosis	*FAS* sequenced
	g/l	pg/ml	U/ml	mg/dl	mg/dl	pg/ml	pg/ml	%	%	%	%		
DGS	1,9	355	1877	81	16	5	59	1,7	45	6,0	2,6	normal	ND
ICOS	ND	ND	ND	ND	ND	<5	209	4,3	83,1	9,0	0,4	normal	DNT
LRBA	4,1	480	9427	84	23	ND	ND	1,5	40,1	5,4	1,6	normal	ND
RALD	13,0	783	1883	66	9	<5	115	2,5	9,9	3,3	1,5	normal	ND
STIM	16,7	960	5979	64	9	<5	166	0,2	4,3	2,8	5,0	normal	ND
X-CGD	ND	ND	ND	ND	ND	ND	ND	2,8	20,1	2,3	2,0	ND	ND
XLP-2	4,2	811	4866	62	19	<5	89	3,0	14,7	6,7	2,7	normal	ND

Supplementary Table 3A

Diagnosis	IgG	Vitamin B12	sCD25	APO-A1	HDL	IL-10	sFASL	DNT	B220	MZB	SMB	Apoptosis	*FAS* sequenced
	g/l	pg/ml	U/ml	mg/dl	mg/dl	pg/ml	pg/ml	%	%	%	%		
CVID	4,7	ND	7126	94	26	47	ND	4,6	ND	10,6	1,3	normal	DNT
CVID	8.5*	ND	6926	ND	6,6	ND	ND	5,8	ND	1,5	0,8	normal	ND
CVID	7,9*	776	1365	105	29	5	301	3,6	8,7	8,8	4,2	normal	ND
CVID	5,2	635	1103	113	44	5	140	1,8	11,0	18,6	2,1	normal	DNT
CVID	6,7*	761	1297	98	27	10	98	1,3	36,7	14,0	0,8	normal	DNT
CVID	6,8*	599	1748	109	32	5	96	2,9	56,1	5,0	2,0	normal	DNT
CVID	6,7*	592	2657	151	49	9	98	2,3	69,4	16,8	0,4	normal	ND
CVID	4,9	832	625	128	45	1	98	1,4	13,7	43,0	4,8	defective	germline
CVID	ND	687	2420	129	43	5	136	2,2	66,4	2,6	0,4	normal	DNT
CVID	9,4*	721	527	126	35	5	99	1,5	5,2	2,0	0,6	normal	ND
CVID	ND	470	1590	123	23	15	111	1,0	10,0	8,1	ND	normal	ND
CVID	0,6	ND	ND	ND	ND	5,5	90	2,0	38,7	3,6	1,4	normal	DNT
CVID	4,5	ND	4610	ND	ND	5	95	4,0	51,3	3,0	0,4	normal	DNT
CVID	8,2*	620	2178	100	34	12	111	ND	65,1	ND	ND	normal	ND
CVID	4,1	480	9427	84	23	ND	ND	1,5	40,1	5,4	1,6	normal	ND
CVID	4,2	845	3779	110	32	ND	ND	1,9	45,0	ND	ND	ND	ND
CVID	7,5*	1291	1134	129	56	5	215	1,8	13,2	3,4	2,5	normal	DNT
CVID	3,0	827	1489	139	68	121	44	1,1	14,0	17,4	2,0	normal	ND
CVID	0,1	629	1645	92	26	5	135	6,6	26,1	15,7	0,5	normal	DNT

Supplementary Table 3B

Supplementary Table 3. Biomarker profile of patients with (A) genetically proven primary immunodeficiencies other than ALPS and (B) CVID. ND, test not done; * under substitution.

	PROBABILITY OF **HAVING** *FAS* MUTATION							
		Current study						NIH study *
		Untreated patients (n=58)		Patients treated with immunosuppression (n=32)		All patients (n=98)		
Parameter	Cut-off (NIH)	Prevalence (%)	PPV (%)	Prevalence (%)	PPV (%)	Prevalence (%)	PPV (%)	PPV (%)
Vitamin B12	> 1500 pg/ml	35	84	10	100	27	87	87
sFASL	> 300 pg/ml	44	83	43	75	44	81	88
IL-10	> 40 pg/ml	45	75	29	75	43	73	85
DNT	> 4 %	36	70	28	44	34	63	89
DNT + vitamin B12	all above cut-off	25	92	7	100	18	93	97
DNT + sFASL	all above cut-off	29	93	14	75	24	90	97
Vitamin B12 + sFASL	all above cut-off	31	94	11	100	25	95	NA
Vitamin B12 + DNT + sFASL	all above cut-off	26	92	7	100	19	93	NA
Vitamin B12 + HDL + APO-A1	As above + HDL > 26 mg/dl + APO-A1 > 92 mg/dl	20	82	7	100	17	86	NA
Vitamin B12 + DNT + HDL + APO-A1	As above + HDL > 26 mg/dl + APO-A1 > 92/mg/dl	17	89	7	100	14	91	NA
Germline *FAS* mutation	present	33	100	25	100	33	100	NA

Supplementary table 4A

		PROBABILITY OF **NOT HAVING** *FAS* MUTATION						
		Current study						NIH study *
		Untreated patients (n=58)		Patients treated with immunosuppression (n=32)		All patients (n=98)		
Parameter	Cut-off (NIH)	Prevalence (%)	NPV (%)	Prevalence (%)	NPV (%)	Prevalence (%)	NPV (%)	NPV (%)
Vitamin B12	< 1000 pg/ml	43	91	66	90	50	91	65
sFASL	< 200 pg/ml	40	95	50	100	43	97	92
IL-10	< 20 pg/ml	49	96	64	83	51	91	67
DNT	< 2 %	32	83	38	67	33	74	76
DNT + vitamin B12	All below cut-off	17	100	28	88	21	94	91
DNT + sFASL	All below cut-off	20	90	18	100	19	93	98
Vitamin B12 + sFASL	All below cut-off	27	100	44	100	33	100	NA
Vitamin B12 + DNT + sFASL	All below cut-off	12	100	19	100	14	100	NA
Vitamin B12 + HDL + APO-A1	As above + HDL < 26 mg/dl + APO-A1 < 92 mg/dl	33	89	59	88	42	88	NA
Vitamin B12 + DNT + HDL + APO-A1	As above + HDL < 26 mg/dl + APO-A1 < 92 mg/dl	11	100	26	86	16	92	NA
Germline *FAS* mutation	absent	67	90	75	92	67	91	NA

Supplementary table 4B

	PROBABILITY OF **HAVING** *FAS* MUTATION						
		Untreated patients (n=58)		Patients treated with immunosuppression (n=32)		All patients (n=98)	
Parameter	Cut-off (current study)	Prevalence (%)	PPV (%)	Prevalence (%)	PPV (%)	Prevalence (%)	PPV (%)
Vitamin B12	>= 1255 pg/ml	44	71	28	75	39	73
sFASL	>=559 pg/ml	40	86	36	80	40	84
IL-10	>= 58 pg/ml	42	73	29	75	40	74
DNT	>= 3.8 %	39	68	34	46	40	61
DNT + vitamin B12	all above cut-off	25	92	14	75	21	88
DNT + sFASL	all above cut-off	29	93	18	80	25	90
Vitamin B12 + sFASL	all above cut-off	33	94	22	83	29	92
Vitamin B12 + DNT + sFASL	all above cut-off	26	92	15	75	22	88
Vitamin B12 + HDL + APO-A1	As above + HDL >= 26 mg/dl + APO-A1 >= 92 mg/dl	17	89	11	67	15	83
Vi tamin B12 + DNT + HDL + APO-A1	As above + HDL >= 26 mg/dl + APO-A1 >= 92 mg/dl	22	75	11	67	20	75

Supplementary table 4C

		PROBABILITY OF **NOT HAVING** *FAS* MUTATION					
		Current study					
		Untreated patients (n=58)		Patients treated with immunosuppression (n=32)		All patients (n=98)	
Parameter	Cut-off (current study)	Prevalence (%)	NPV (%)	Prevalence (%)	NPV (%)	Prevalence (%)	NPV (%)
Vitamin B12	< 1255 pg/ml	56	90	72	86	61	88
sFASL	< 559 pg/ml	60	94	64	94	60	94
IL-10	< 58 pg/ml	59	87	71	85	60	87
DNT	< 3.8 %	61	79	66	76	60	77
DNT + vitamin B12	All below cut-off	42	96	55	88	46	92
DNT + sFASL	All below cut-off	47	92	46	92	47	92
Vitamin B12 + sFASL	All below cut-off	46	96	56	100	49	97
Vitamin B12 + DNT + sFASL	All below cut-off	35	94	41	100	37	97
Vitamin B12 + HDL + APO-A1	As above + HDL < 26 mg/dl + APO-A1 < 92 mg/dl	28	93	52	86	36	90
Vitamin B12 + DNT + HDL + APO-A1	As above + HDL < 26 mg/dl + APO-A1 < 92 mg/dl	35	90	59	81	43	86

Supplementary table 4D

Supplementary Table 4.: Positive and negative predictive values for all tested biomarkers and biomarker combinations in patients with or without immunosuppressive treatment.

(4A, 4B) Positive predictive values (PPV) and negative predictive values (NPV) for having a *FAS* mutation for the relevant biomarkers and their combinations using cut-offs defined by Caminha et al. (NIH study, (11)) and (4C,4D) same as above using our cut-offs based on *FAS* mutation prevalence in our cohort. Prevalence in our cohort, PPV and NPV are shown for untreated patients, patients treated with immunosuppressive drugs and all patients (treated, untreated and all). NA, not available; * data on prevalence unknown.