Pre-existing antibodies against polyethylene glycol reduce asparaginase activities on first administration of pegylated *E. coli* asparaginase in children with acute lymphocytic leukemia

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Supplementary data

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Reported prevalence of antibodies against PEG

Various studies examined the occurrence of polyethylene glycol (PEG) antibodies among healthy humans as well as patients treated with pegylated drugs. Tables S1 and S2 summarize the reported prevalence of antibodies against PEG in healthy subjects (Table S1) and patients treated with pegylated drugs (Table S2) and provide information on the methods used for the detection of antibodies against PEG.

Table S1: Studies reporting the prevalence of anti-PEG antibodies in healthy individuals [1–7]

Reference	Richter et al. 1984[1]		Leger et al. 2001[2]	Armstrong et al. 2003[3]	Liu et al. 2011[4]	Lubich et al. 2016[5]		Chen et al. 2016[6]	Yang et al. 2016[7]
No. of participants (n)	453		100	250	350	710	600	1504	377
Origin (n)	JPN (142), GER (151), ITA (169)		N/R	N/R	N/R	AUT (710)	AUT (100), USA (500)	TWN (1504)	CAU (200), AFR (49), HIS (42), ASIA (37)
Age [years]	N/R[a]		N/R	N/R	N/R	range: 18 - 67		mean±SD: 52.1 ± 17.4	≤ 70
Sex (m/f)	N/R		N/R	N/R	N/R	N/R	N/R	756 / 748	226 / 151
Method	Hemagglutination		Hemagglutination	Hemagglutination (HA), Flow Cytometry (FC)	double antigen bridging ELISA	Flow Cytometry	ELISA	ELISA	ELISA
Antigen	methoxy-PEG (3 kDa)		amine-PEG	HA: mPEG (20 kDa) FC: PEG (TentaGel-OH^{TM} particles)	PEG (40 kDa)	PEG (TentaGel-OH^{TM} particles)	methoxy-PEG (20 kDa, branched) bound to serumalbumin	amine-PEG (10 kDa)	methoxy-PEG (5 kDa) bound to 1,2 distearoyl-sn-glycero-3-phospho-ethanolamine
Cut Point Definition	Agglutination		Agglutination	Agglutination	meanODs ± 1.645 x SD	IgG/IgM background signal levels[c]	IgG background signal levels[c] IgM daily floating cut-point	> 3x mean ODs of background	> corrected OD of the lowest standard
Prevalence									
anti-PEG AB	**4.7%** (21/453) (for titers > 2 and	**0.2%** (1/453) (for titers ≥32)	**22%** (22/100)	HA: **25%** (62/250)	**4.3%** (15/350)	**23%** (163/710)	**24%** (144/600)	**44.3%** (666/1504)	**72%** (273/377)
anti-PEG-IgG	N/A[b]	N/A	N/A	FC: **18.4%** (21/250)	N/A	**13%** (92/710)	**14%** (84/600)	**25.7%** (386/1505)	**47.7%** (180/377)
anti-PEG-IgM	N/A	N/A	**22%** (22/100)	FC: **3.6%** (9/250)	N/A	**15%** (107/710)	**12%** (72/600)	**27.1%** (407/1504)	**54.6%** (206/377)
anti-PEG IgG + anti-PEG IgM	N/A	N/A	N/A	FC: **3.2%** (8/250)	N/A	N/R	N/R	**8.4%** (126/1504)	**30%** (113/377)

[a] N/R - not reproted; [b] N/A- not analyzed; [c] - background signal levels were determined in healthy human volunteers

Table S2: Studies reporting on the prevalence and the effects of anti-PEG antibodies on the efficacy of pegylated drugs[1,3,8–12]

Reference	**Richter et al. 1984[1]**		**Ganson et al. 2006[8]**	**Armstrong et al. 2007[9]**		**Tillmann et al. 2010[10]**	**Hershfield et al. 2014[11]**	**Myler et al. 2015[12]**		**Ganson et al. 2006[13]**	
Disease/ Treatment	Hyposensitisation		Gout	ALL		Hepatitis	Gout	Hepatitis		Anitcoagulation	
Drug	pegylated allergens		PEG-uricase	PEG-ASNase		PEG-interferon	PEG-uricase	PEG-interferon λ	PEG-interferon α	Pegnivacogin	
No of patients (n)	92		13	28		68	30	32	22	354.00	
Age [years]	N/R[a]		range: 40 - 75	mean: 8.3		N/R	range: 25 - 93	N/R	N/R	N/R	
Sex (m/f)	N/R		10 / 3	17 / 11		N/R	8 / 22	N/R	N/R	N/R	
Method	Hemagglutination		direct ELISA	Hemagglutination	Flow cytometry	ELISA	ELISA	bridged ELISA using Meso Scale Discovery Technology		direct and competition ELISA	
Antigen	methoxy-PEG (3kDa)		PEG-uricase (vs uricase)	methoxy-PEG (20 kDa)	PEG (TentaGel-OH™ particles)	PEG-uricase vs PEG (10 kDa)	methoxy-PEG (10 kDa)	PEG-Inf λ vs branched PEG (40 kDa)	PEG-Inf α vs branched PEG (40 kDa)	PEG-uricase (vs uricase)	mPEG component of pegnivacogin (40 kDa)
Cut Point Definition	Agglutination		> mean absorption of healthy control sera + 3SD	Agglutination	FI > 100 (anti-PEG IgG) FI > 50 (anti-PEG IgM)	mean + 3x SD	mean + 3x SD	mean + 1.645x SD	mean + 1.645x SD	N/R	
Prevalence before drug exposure											
anti-PEG AB	**17.3%** (16/90) (for titers > 2 and	**3.3%** (3/90) (for titers ≥32)	N/A	N/A	N/A	**44%** (30/68)	**26.6%** (8/30)	**6.3%** (2/32)	**9.1%** (2/22)	N/A	N/A
anti-PEG-IgG	N/Ab	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**36%** (128/354) (for A405 > 0.15)	**23%** (82/354) (for A405 >0.2)
anti-PEG-IgM	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
anti-PEG IgG + anti-PEG IgM	N/A	N/A	N/A	N/A	NE/	N/A	N/A	N/A	N/A	N/A	N/A
Prevalence after drug exposure											
anti-PEG AB	**27.5%** (25/90) (for titers >2 and <32)	**50%** (45/90) (for titers ≥32)	N/A	**32%** (9/23)	N/A	N/A	**43%** (13/30)	**25%** (8/32)	**27%** (6/22)	N/A	N/A
anti-PEG-IgG	N/A	N/A	**39%** (5/13)	N/A	**32%** (9/23)	N/A	N/A	N/A	N/A	N/A	N/A
anti-PEG-IgM	N/A	N/A	N/A	N/A	**46%** (13/23)	N/A	N/A	N/A	N/A	N/A	N/A
anti-PEG IgG + anti-PEG IgM	N/A	N/A	N/A	N/A	**32%** (9/23)	N/A	N/A	N/A	N/A	N/A	N/A
Effects on treatment	N/A		loss of PEG-uricase efficacy	loss of PEG-ASNase activity		no effect	loss of PEG-uricase efficacy	no hypersensitivity reactions to PEG-Inf λ	no hypersensitivity reactions to PEG-Inf α	severe first exposure hypersensitivity reactions to Pegnivacogin	

[a] N/R - not reproted; [b] N/A - not analyzed; [c] - background signal levels were determined in healthy human volunteers

Methods

Patients

Samples for anti-PEG antibody determination were obtained from two cohorts of children and 1 cohort of adults with ALL and from 1 cohort of healthy infants, which served as the reference cohort. Further details about the ALL-cohorts are provided below:

Children with primary ALL – ALL-cohort 1

Patients of ALL-cohort 1 were diagnosed with primary ALL and treated according to the AIEOP-BFM ALL 2009 trial (ClinicalTrials.gov identifyler: 01117441). A total of 673 plasma samples collected from 673 pediatric patients (401 males, 272 females) prior to their first administration of PEG-ASNase were analyzed for IgG and IgM antibodies against PEG. 646 patients additionally provided 1-2 serum samples (1183 in total) taken within 15 days after the first PEG-ASNase dose on day 12 of induction.

In the AIEOP-BFM ALL 2009 protocol, the first dose of PEG-ASNase is administered on day 12 in induction. Additional chemotherapy received prior to first PEG ASNase dose included corticoids (from day 1), intrathecal methotrexate (on day 1), daunorubicin (on day 8) and vincristine (on day 8). Some patients with T-ALL received an additional infusion of cyclophosphamide on day 10 (2 days prior to PEG-ASNase). PEG-ASNase was applied without any premedication with hydrocortisone, diphenhydramine or acetaminophen.

Children with relapsed ALL – ALL-cohort 2

Patients of ALL-cohort 2 were diagnosed with relapsed ALL and treated according to the protocol of the ALL-REZ BFM 2002 (ClinicalTrials.gov identifyler: 00114348) or the

ALL-REZ BFM Observational study and Biobank study. If patients tolerated PEG-ASNase during primary ALL treatment they continued with PEG-ASNase for relapse treatment. A total of 28 serum samples were available from 28 patients (19 males, 9 females). Since the ALL-REZ BFM 2002 study scheduled monitoring of antibodies and PEG-ASNase activity after PEG-ASNase administration, the analyzed samples were collected 0 to 2 days after the first PEG-ASNase dose. Before their first PEG-ASNase on day 4 of the F1 block relapse patients were treated with dexamethasone since the start of F1 and received one infusion of vincristine on day 1 and a 36h-infusion of methotrexate starting on day 1.

Adults with primary ALL – ALL-cohort 3

Patients in ALL-cohort 3 were adult patients with ALL treated according to the multicenter GMALL 07/2003 trial (ClinicalTrials.gov identifyler: 00198991). A total of 188 samples from 120 males and 68 females were analyzed for anti-PEG IgG and IgM antibodies. Like in the ALL-REZ BFM 2002 study antibodies and PEG-ASNase activities were monitored after PEG-ASNase administration. The samples were taken on the same day after the first administration of PEG-ASNase (n=16) or the following day (n=172). In the GMALL 07/2003 protocol the first PEG-ASNase was prescribed on day 20 of induction treatment. Until then, patients had intermittently received dexamethasone on day 1 to 7 and day 13 to 16, three injections of vincristine on days 6, 13, 20, three infusions of cyclophosphamide on days 3, 4, and 5. Patients with Ph/BCR-ABL negative ALL received 4 infusions of daunorubicin on days 6, 7, 13, and 14. Patients with more than 20% of their blasts stained positive for CD20 received rituximab prior to phase I. Ph/BCR-ABL positive ALL patients received imatinib but no daunorubicin or rituximab.

Determination of antibodies against PEG

For the detection of anti-PEG antibodies we have transferred the flow cytometric method developed by Armstrong et al. to a 96-well plate format with a fluorescent read-out.[3]

TentaGel M OCH_3 particles (10 µm), to which methoxy-polyethylene glycol chains with a mean molecular weight of 5000 Da were covalently bound, were used as antigen (RAPP Polymere, Tuebingen, Germany). A 100 µL suspension of 0.1% TentaGel M OCH_3 particle suspension in PBS-buffer was added to 25 µL serum and incubated for 30 minutes at room temperature. Subsequently, 100 µL of PBS were added and the suspension was centrifuged at 2000 RCF for 2 minutes. The supernatant was discarded and the remaining particles were washed 3 times with PBS-buffer. Then 100 µL of PBS-solution containing 1 µL of fluorescein-labeled anti-human IgG (Sigma-Aldrich, Steinheim, Germany) and 0.2 µL of R-phycoerythrin labeled anti-human IgM (Biozol Diagnostica Vertrieb GmbH, Eching, Germany) were added to the pellet. Afterwards the pellet was carefully resuspended and incubated for 1 h at room temperature in the dark. The particles were again washed 3 times with PBS-buffer. Finally, the fluorescence intensities of the bound secondary anti-human IgG/IgM were determined with a Fluoroscan Ascent FL (Thermo Fisher Scientific, Braunschweig, Germany). Each sample was analyzed in duplicate and the mean fluorescence intensity (MFI) was calculated for each sample. No Tween™, which has been reported to interact with the detection of anti-PEG antibodies, was added in any step.[5]

Samples were categorized as positive or negative according to their MFI. Samples with high MFI (>25 for anti-PEG IgG and >10 for anti-PEG IgM) and low MFI (<5 for anti-PEG IgG and <1 for anti-PEG IgM) were used for preparation of quality control

samples. The respective samples were pooled, analyzed independently in different runs and based on means and standard deviations acceptance criteria were defined. Quality control samples with high and low MFI were included in each run and a run was discarded, if the MFI of the positive and negative control samples deviated by more than 2 standard deviations (S.D.) from the previously determined means of the quality controls. The assay showed acceptable reproducibility with intraday precision below 10% for anti-PEG IgG and anti-PEG IgM. Inter-day precision for anti-PEG IgG and IgM measurements was below 5% for samples with high MFI ($MFI_{anti\text{-}PEG\ IgG}$ >25; $MFI_{anti\text{-}PEG\ IgM}$: >10) and below 14% for samples with low MFI ($MFI_{anti\text{-}PEG\ IgG}$ <5; $MFI_{anti\text{-}PEG\ IgM}$: <1).[13]

Definition of cut-points for anti-PEG IgG and anti-PEG IgM

The MFI, determined for anti-PEG IgG and IgM in samples of the reference cohort, were used to define cut-points, which could classify samples as positive or negative. Means of anti-PEG IgG and IgM levels of the reference cohort plus 1.645 times the standard deviation, which represented the upper 95% confidence interval, were calculated as described by Mire-Sluis *et al.*[14] In the reference cohort the upper 95% CI was 5.01 for anti-PEG IgG MFI and 1.06 for anti-PEG IgM MFI. After an additional visual adjustment a MFI of 8 was defined as cut-point for anti-PEG IgG and a MFI of 2 as cut-point for anti-PEG IgM for a better usability. Samples with $MFI_{anti\text{-}PEG\ IgG}$ >8 and with $MFI_{anti\text{-}PEG\ IgM}$ >2 were considered positive for anti-PEG IgG and anti-PEG IgM, respectively. The MFI of 8 of the FITC-labeled anti-human IgG antibody corresponded to a protein concentration of 0.05 µg/ml, the MFI of 2 of the R-phycoerythrin conjugated anti-human IgM antibody to a protein concentration of 0.2 µg/ml.

Evaluation of assay performance for the prediction of hypersensitivity reactions to PEG-ASNase

Among the 673 children in ALL-cohort 1, 110 were high-risk patients at risk for hypersensitivity reactions to PEG-ASNase.[15] Of these 110 patients, 21 patients developed hypersensitivity reactions against PEG-ASNase after the 3rd to 5th PEG-ASNase administration (2500 U/m^2; maximum: 3750 U per dose). Antibody status was analyzed in serum samples collected prior to administration of PEG-ASNase. The distribution of anti-PEG antibody positive/negative samples collected immediately prior to PEG-ASNase administrations with hypersensitivity reactions was compared to that of patients without hypersensitivity reactions. Of 21 patients, who developed hypersensitivity reactions against PEG-ASNase, 17 patients were positive for anti-PEG IgG and 11 for anti-PEG IgM before hypersensitivity reactions. In this group the following performance characteristics were determined for anti-PEG IgG (> 8 MFI) and IgM (> 2 MFI) to predict hypersensitivity reactions to PEG-ASNase:

anti-PEG IgG		**anti-PEG IgG**	
sensitivity:	81.0%	sensitivity:	52.4%
specificity:	97.9%	specificity:	95.9%
positive predictive value:	85.0%	positive predictive value:	64.7%
negative predictive value:	97.3%	negative predictive value:	93.4%

The detection of anti-PEG antibodies immediately prior to PEG-ASNase predicted the occurrence of hypersensitivity reactions with satisfactory sensitivity and specificity. It has to be noted that the shorter the interval between antibody detection and the occurrence of the hypersensitivity reactions, the better the performance of the antibody test.[16]

Measurement of ASNase activity

PEG-ASNase activities were determined by hydrolysis of aspartic acid-ß-hydroxamate as described previously.[17] The lower limit of quantification (LLOQ) was 5 U/L. The assay showed acceptable reproducibility and accuracy with an inter-day precision <6% and an accuracy ≤12% within a calibration range between 5 and 1000 U/L PEG-ASNase.

Measurement of total IgG and IgM

Total IgG and IgM were measured in human serum by an immunoturbidimetric assay on a cobas c502 chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The intra- and inter-assay coefficients of variation were <5% for both tests.

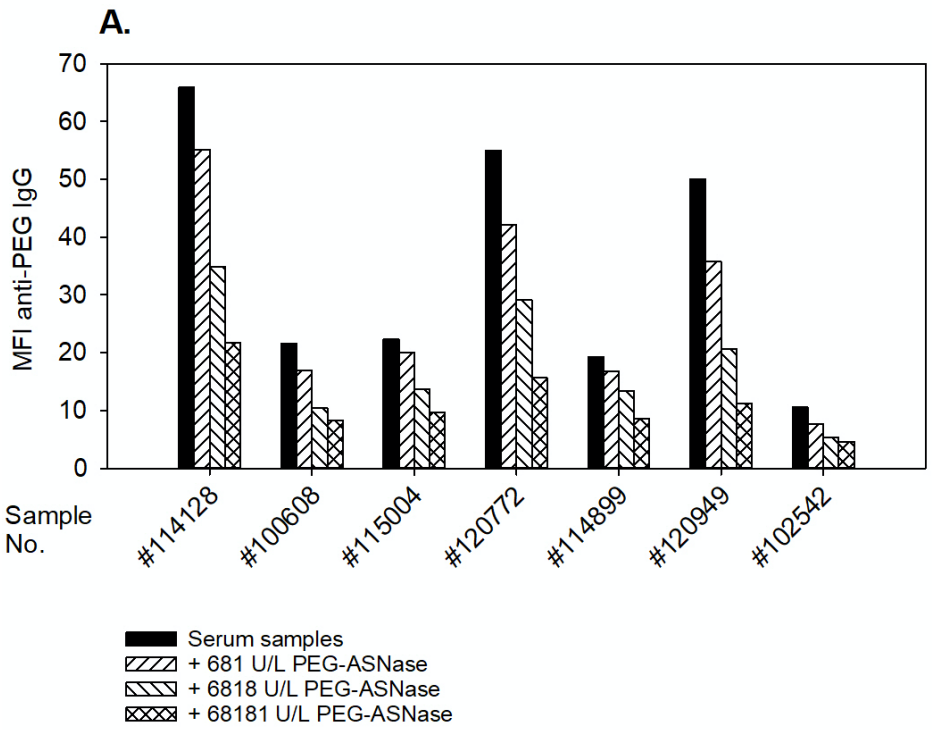
Competition experiments

To confirm the specificity of the detected antibodies to PEG and to minimize false positives competition assays were performed using PEG-ASNase and PEG 5000 Da as competing antigens.[5,6] Serum samples with anti-PEG IgG MFI >20 and/or anti-PEG IgM MFI >10 were spiked with PEG-ASNase (Oncaspar™, Servier, Suresnes, France), PEG 5000 (Sigma Aldrich, Deisenhofen, Germany), or antigen-free PBS solution and incubated at 37°C for 45 min, before they were analyzed for anti-PEG IgG and anti-PEG IgM. 5 µL Oncaspar™ solution (750 U/ml) or 5 µL PEG 5000 (0.5 mg/ml) was added to 50 µL serum (final concentration: PEG-ASNase concentration: 68181 U/L; PEG 5000 concentration 455 mg/L). Lower PEG-ASNase and PEG 5000 concentrations were prepared by 1:10 dilutions. For the inhibition of PEG-ASNase activity samples with anti-PEG IgG MFI >20 and known amounts of PEG-ASNase were spiked with PEG-ASNase, incubated at 37°C for 45 min and analyzed for PEG-

ASNase activity. The recovery was calculated from the difference between the expected (known + spiked) PEG-ASNase activity and the determined PEG-ASNase activity. Samples with anti-PEG IgG levels <5 MFI served as controls.

Both antigens - PEG-ASNase as well as PEG 5000 - were able to reduce the MFI of anti-PEG IgG in a dose dependent manner (Figure S1). The addition of PEG-ASNase and/or PEG 5000 antigen was able to reduce the MFI of anti-PEG IgM, but the effects were less consistent compared to the reductions observed for anti-PEG IgG (Figure S2). IgM are known to bind to target structures with lower affinity, because they lack significant selection by somatic hypermutation.[18] This might explain the more heterogeneous results of the competition experiments in samples with high levels of anti-PEG IgM compared to samples with high levels of anti-PEG IgG.[19]

Figure S1: Mean fluorescence intensity (MFI) determined for anti-PEG IgG after addition of different concentrations of the PEG-ASNase antigen (A.) and the PEG 5000 antigen (with or without native E. coli ASNase) (B.).



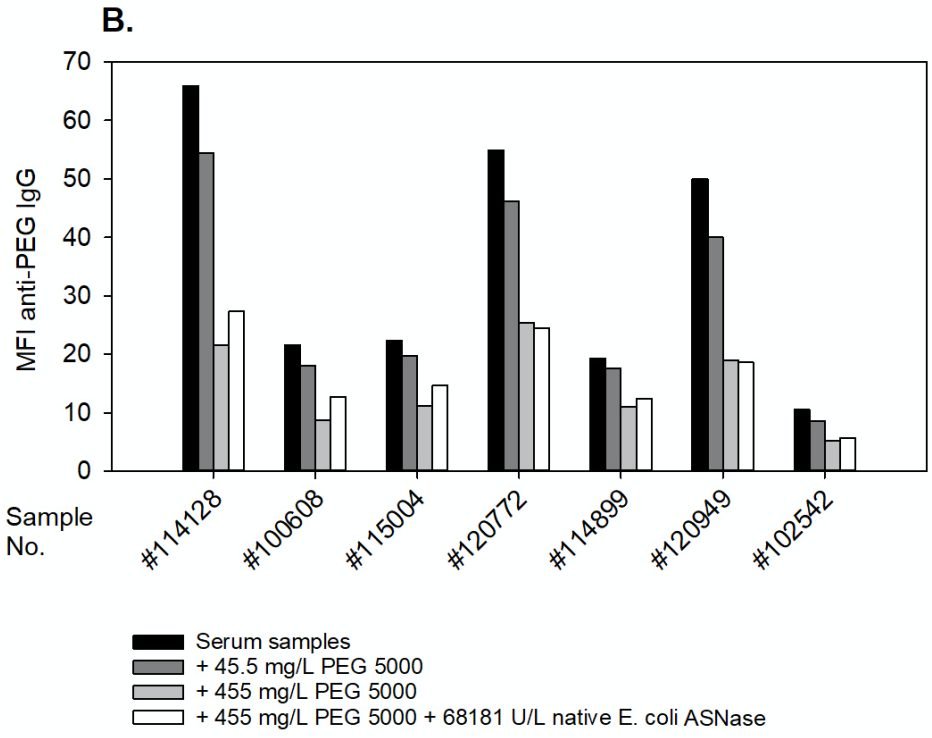
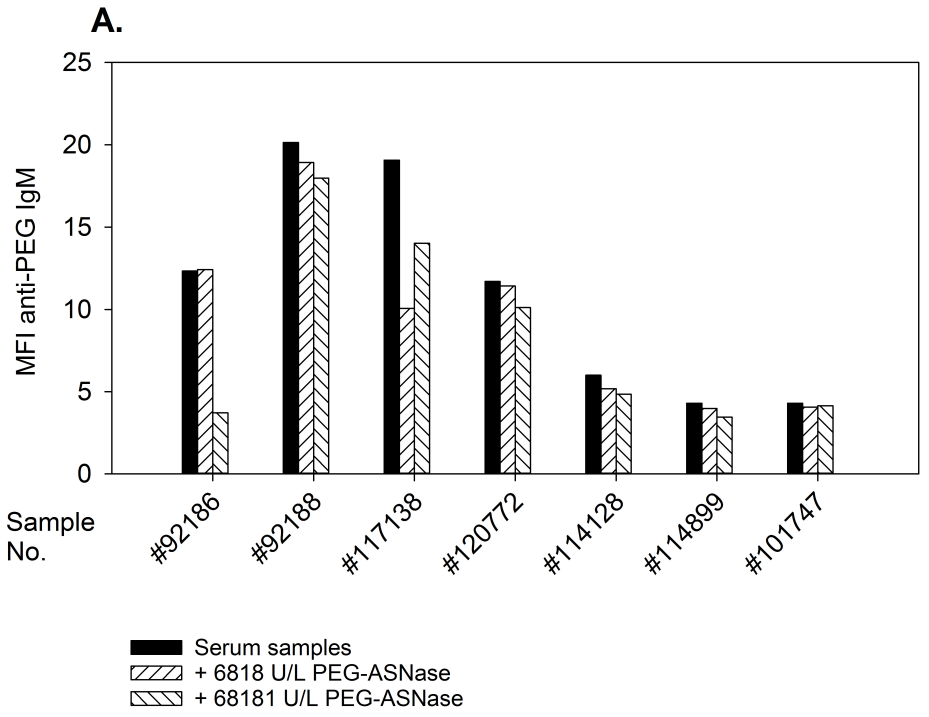
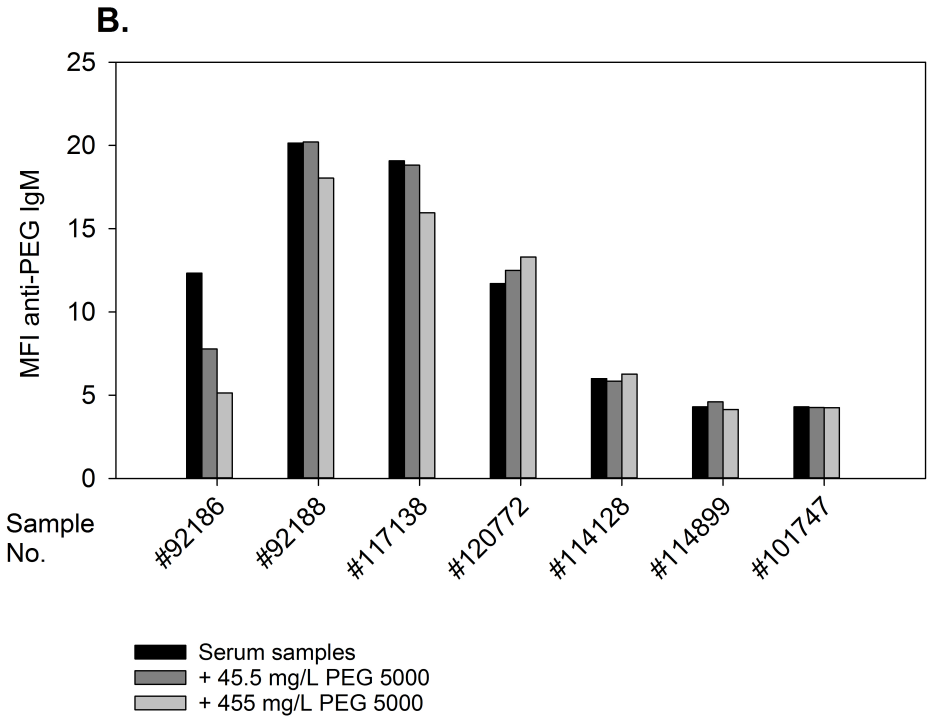


Figure S2: Mean fluorescence intensity (MFI) determined for anti-PEG IgM after addition of different concentrations of the PEG-ASNase antigen (A.) and the PEG 5000 antigen (with or without native E. coli ASNase) (B.).





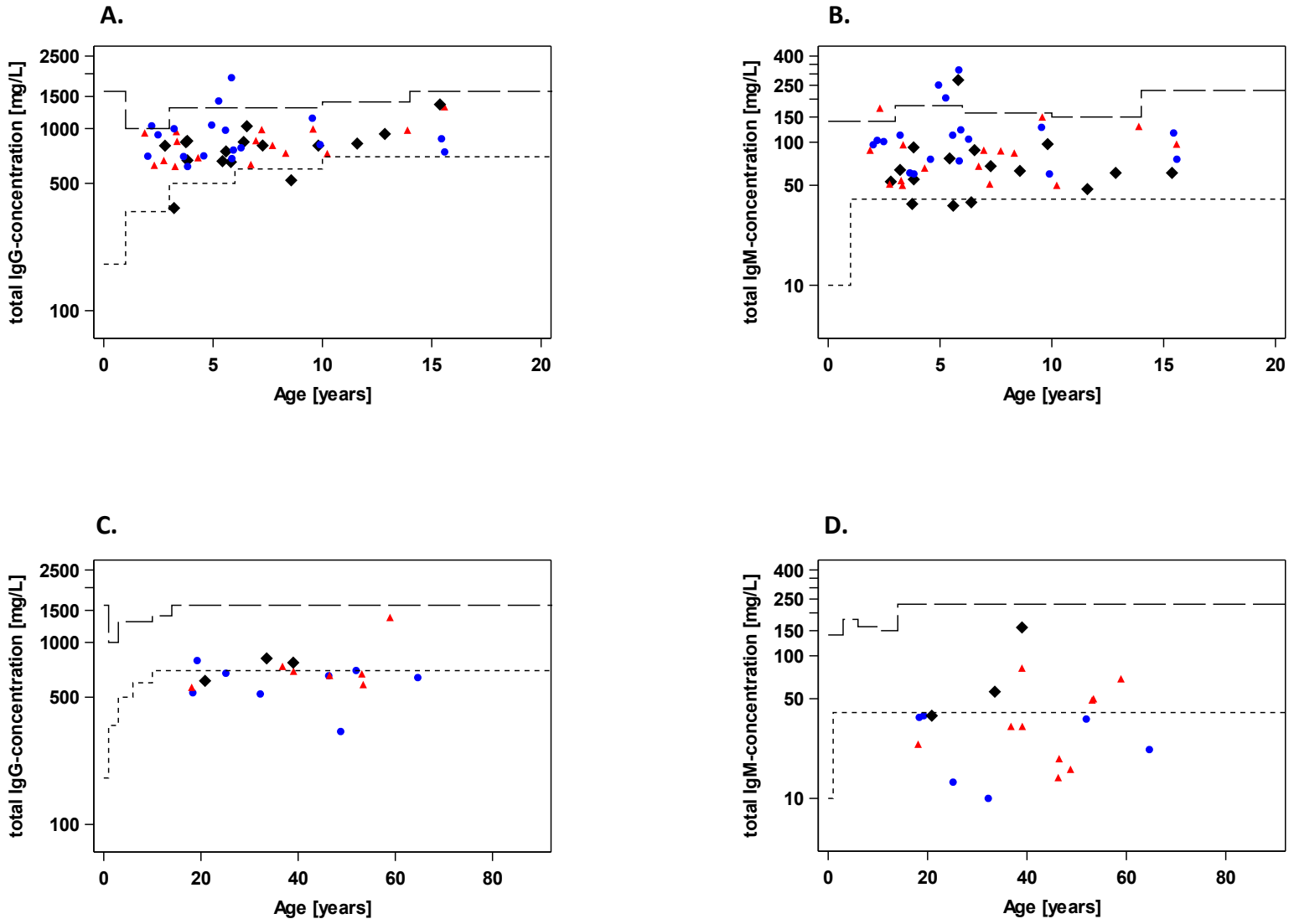
Interferences with total IgG and IgM

Total immunoglobulin concentrations were determined in a subset of 50 samples from ALL-cohort 1 and 19 samples from ALL-cohort 3. Samples with high MFI for anti-PEG IgG and IgM ((MFI ≥ 50th percentile), intermediate MFI (MFI within the 25th-50th percentile) and low MFI (MFIs<25th percentile) were selected.

Total IgG concentrations were within or below the age-dependent normal ranges for 97.5% of patients and total IgM concentrations were within or below the age-dependent ranges for 95% of patients (Figure S3). Exceptional high anti-PEG IgG levels (> 95th percentile) were not significantly associated with high total IgG levels above the respective age range. The same applied for high anti-PEG IgM levels and total IgM concentrations (p = 1.0, exact Fisher test for IgG and IgM).

Figure S3: Total IgG and IgM levels in 69 selected samples from ALL-cohort 1 (A+B) and ALL-cohort 3 (C+D). The lines show the normal concentration range of total IgG and IgM at the respective age (source: central laboratory of the university hospital of Muenster). The black diamond (♦) represents MFI values of anti-PEG IgG and anti-PEG IgM, which were above the 95th percentile; the red triangle (▲) represents MFI values of anti-PEG IgG and anti-PEG IgM between the 25th percentile and the 50th percentile; the blue dot (●) MFI values of anti-PEG IgG and anti-PEG IgM below the 25th percentile.

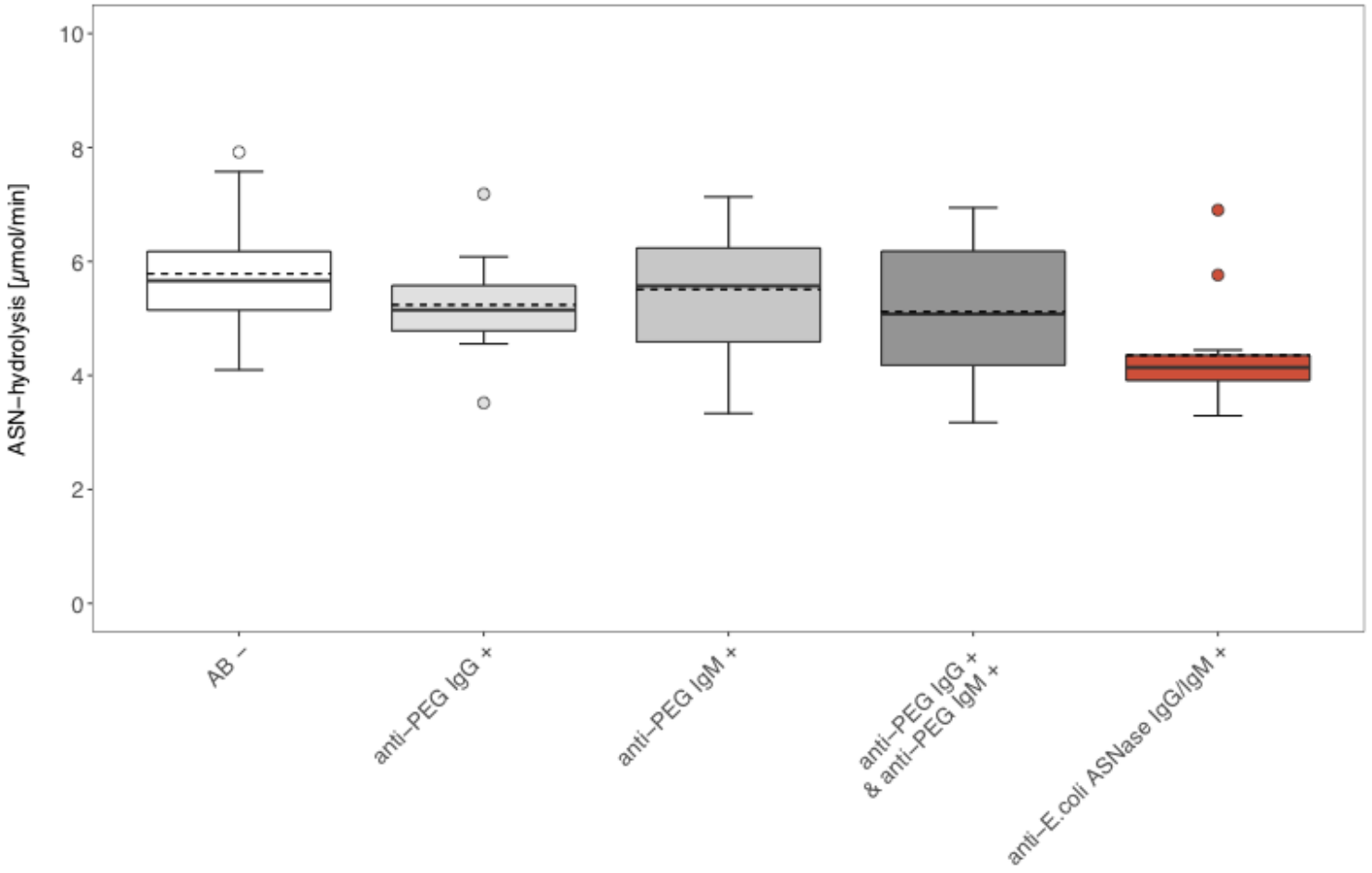
Figure S3:


A.
total IgG-concentration [mg/L]
2500
1500
1000
500
100
0
5
10
15
20
Age [years]
B.
total IgM-concentration [mg/L]
400
250
150
100
50
10
0
5
10
15
20
Age [years]
C.
total IgG-concentration [mg/L]
2500
1500
1000
500
100
0
20
40
60
80
Age [years]
D.
total IgM-concentration [mg/L]
400
250
150
100
50
10
0
20
40
60
80
Age [years]

Evaluation of asparagine hydrolysis in the presence of anti-PEG IgG, anti-PEG IgM and anti-E.coli ASNase

To evaluate whether of antibodies against PEG or native E.coli ASNase were able to inhibit PEG-ASNase activity 54 serum samples were selected, which were either highly positive for anti-PEG IgG (n=8), or anti-PEG IgM (n=9), or both (n=12), or positive for anti-E.coli ASNase IgG/IgM (n=13), or antibody negative (n=12). Anti-E.coli ASNase IgG/IgM were determined as previously described.[20] No ASNase activity was detectable in any samples. All samples were analyzed for asparagine and aspartic acid by HPLC as previously described.[21] PEG-ASNase was added to each sample (final concentration: 10 U/L). Samples were incubated at 37°C. After 10 min the reaction was stopped with sulphosalicylic acid and asparagine and aspartic acid were determined in each sample. Substrate turn-over was calculated as hydrolysis of asparagine per min.

Figure S4: Rate of asparagine (ASN) hydrolysis by PEG-ASNase (10 U/L) in the absence or presence of anti-PEG or anti-E.coli ASNase antibodies.



Specificity of the detection of anti-PEG IgG and IgM

Serum samples of ALL-cohort 1 were also analyzed for antibodies against native E. coli ASNase as previously described.[20] The fact that samples were identified, which were exclusively positive for anti-PEG IgG (Table S3), anti-PEG IgM (Table S4) or anti-E. coli ASNase IgG/IgM (Table S5)), shows that the method can in principle distinguish between anti-PEG IgG and anti-PEG IgM. In case the samples were positive for anti-PEG IgG and anti-PEG IgM, interferences per se cannot be excluded.

Table S3: IgG/IgM against native E. coli ASNase and anti-PEG IgM determined in samples classified as positive for anti-PEG IgG

Sample No.	IgG/IgM against native E. coli ASNase[a]		anti-PEG IgG		anti-PEG IgM	
	S/C value[b,c]	category	MFI[d]	category	MFI[e]	category
100984	0.09	-	34.64	+	1.02	-
92104	0.03	-	34.55	+	1.90	-
114173	0.26	-	34.21	+	1.57	-
100983	0.11	-	34.07	+	1.09	-
98084	0.89	-	32.30	+	1.09	-
90224	0.32	-	31.90	+	0.96	-
98358	0.05	-	31.17	+	0.98	-
90234	0.29	-	31.16	+	0.89	-
93876	0.05	-	30.84	+	1.48	-
93170	0.63	-	30.70	+	0.83	-

[a] - IgG/IgM antibodies against native E. coli ASNase were analyzed by the medac company[20]
[b] - S/C (sample control ratio) values were calculated by dividing the OD of the sample through a cut off determined individually for each analysis run
[c] - S/C values were categorized as follows: S/C <0.9: "-"; S/C 0.9-1-1: "±"; S/C >1.1-2.5: "+"; S/C >2.5-13.5: "++", S/C >13.5: "+++"
[d] - MFI (mean fluorescent intensity) values were categorized as follows: MFI ≤8: "-"; MFI >8: "+"
[e] - MFI values were categorized as follows: MFI ≤2: "-"; MFI >2: "+"

Table S4: IgG/IgM against native E. coli ASNase and anti-PEG IgG determined in samples classified as positive for anti-PEG IgM

Sample No.	IgG/IgM against native E. coli ASNase[a]		anti-PEG IgG		anti-PEG IgM	
	S/C value[b,c]	category	MFI[d]	category	MFI[e]	category
92175	0.50	-	7.47	-	9.62	+
115691	0.12	-	7.32	-	8.05	+
99367	0.40	-	7.98	-	7.99	+
88080	0.20	-	7.01	-	7.90	+
97043	0.07	-	6.40	-	7.35	+
79092	0.14	-	7.58	-	6.99	+
89879	0.41	-	6.47	-	6.96	+
109458	0.14	-	6.85	-	6.95	+
106939	0.47	-	7.21	-	6.86	+
88277	0.05	-	5.77	-	6.49	+

[a] - IgG/IgM antibodies against native E. coli ASNase were analyzed by the medac company[20]
[b] - S/C (sample control ratio) values were calculated by dividing the OD of the sample through a cut off determined individually for each analysis run
[c] - S/C values were categorized as follows: S/C <0.9: "-"; S/C 0.9-1-1: "±"; S/C >1.1-2.5: "+"; S/C >2.5-13.5: "++", S/C >13.5: "+++"
[d] - MFI (mean fluorescent intensity) values were categorized as follows: MFI ≤8: "-"; MFI >8: "+"
[e] - MFI values were categorized as follows: MFI ≤2: "-"; MFI >2: "+"

Table S5: Anti-PEG IgG and IgM determined in samples classified as positive for IgG/IgM against native E. coli ASNase

Sample No.	IgG/IgM against native E. coli ASNase[a]		anti-PEG IgG		anti-PEG IgM	
	S/C value[b,c]	category	MFI[d]	category	MFI[e]	category
110614	18.32	+++	3.07	-	0.46	-
110613	14.70	+++	2.03	-	0.40	-
110612	14.58	+++	4.44	-	0.42	-
102140	12.92	++	3.27	-	1.15	-
105224	12.80	++	4.77	-	0.81	-
105260	12.11	++	4.16	-	0.71	-
105196	11.59	++	6.17	-	1.00	-
102472	11.58	++	3.15	-	0.26	-
105261	11.38	++	3.78	-	0.76	-
102636	11.05	++	4.41	-	0.55	-

[a] - IgG/IgM antibodies against native E. coli ASNase were analyzed by the medac company[20]
[b] - S/C (sample control ratio) values were calculated by dividing the OD of the sample through a cut off determined individually for each analysis run
[c] - S/C values were categorized as follows: S/C <0.9: "-"; S/C 0.9-1-1: "±"; S/C >1.1-2.5: "+"; S/C >2.5-13.5: "++", S/C >13.5: "+++"
[d] - MFI (mean fluorescent intensity) values were categorized as follows: MFI ≤8: "-"; MFI >8: "+"
[e] - MFI values were categorized as follows: MFI ≤2: "-"; MFI >2: "+"

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