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Novel mutation in DOCK8-HIES with Severe Phenotype and Successful Transplantation

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Abstract:

Background: Hyper-IgE syndrome (HIES) due to DOCK8 deficiency is an autosomal recessive (AR) primary combined immunodeficiency which results in significant morbidity and mortality at a young age. Different mutations in the DOCK8 gene can lead to variable severity of the disease.

Objective: We evaluated the genetic mutations in three related patients with severe clinical manifestations suggestive of AR HIES. We also explored whether treatment with stem cell transplantation could lead to complete disease resolution.

Method: We examined the clinical manifestations and immunological workup of these patients. Their DNA was also screened for causative mutation. Post transplantation, clinical and immunological data for the transplanted patient was also collected.

Results: All patients had a severe course of the disease with rarely reported severe complications in HIES. One patient died with lymphoma while another died with progressive multifocal leukoencephalopathy (PML) due to a slow virus. All our patients had two novel mutations in the DOCK8 gene. One of these mutations was a novel pathogenic mutation and explains the severity of the disease (homozygous splice site mutation at position 5 after the end of exon 45), while the other mutation was mostly non-pathogenic. Hematopoietic stem cell transplantation (HSCT) was performed in the youngest patient with excellent engraftment and full reversibility of the clinical manifestations.

Conclusion: We report 3 patients from a consanguineous family diagnosed with AR-HIES due to a novel pathogenic mutation in DOCK8 gene leading to fatal outcome in 2 patients and complete resolution of the clinical and immunological features in the third patient by HSCT.

Clinical Importance:

- DOCK8-HIES is a rare disease caused by mutation in a large gene.
- The correlation between a mutation and severity was explored in this report.
- HSCT is the only definite treatment modality which can lead to complete resolution of the disease especially if performed early enough to avoid the development of severe complications.

- **Key words:** Hyper-IgE syndrome, DOCK8 deficiency, Novel mutations, Progressive multifocal leukoencephalopathy, Slow virus, Adrenal Epstein–Barr virus (EBV)-related Leiomyoma.

Introduction:

Hyper-IgE syndrome (HIES), was first described in 1966 by Davis(1). It is a primary combined immunodeficiency characterized by atopic dermatitis, recurrent skin and deep seated infections, many atopic manifestations like food allergy and asthma, and a markedly increased level of serum IgE. To date, four mutations have been described to cause HIES; the signal transducer and activator of transcription 3 (STAT3), the signaling (Autosomal dominant, AD), dedicator of cytokinesis 8 (DOCK8), the signaling (Autosomal recessive, AR), tyrosine kinase 2 (TYK2) signaling alterations (AR) and lastly, reported in 2014, phosphoglucomutase 3 (PGM3) mutations (AR) (2-7)

Patients suffering from HIES due to DOCK-8 mutation suffer from a severe phenotype with higher mortality at a younger age (8). They are prone to cutaneous, pulmonary and deep seated infections due to bacterial, fungal, mycobacterial and viral infections including the human papilloma virus (HPV), herpes zoster, disseminated varicella and molluscum contagiosum (9, 10). In addition, they have a higher tendency to develop autoimmunity and malignancies including lymphomas and squamous cell carcinomas(11).

In this report, we discuss three related Saudi patients from a highly consanguineous family with a novel mutation of the DOCK 8 gene leading to severe clinical course and fatal complications. We also report successful allogeneic bone marrow transplantation in one of the family members with an excellent outcome.

Patients and Clinical presentations:

Patient P1:

A male patient from a highly consanguineous family (Figure 1) was followed in an immunology clinic since the age of two years for severe eczematous skin rash, recurrent chest infections, and chronic otitis media. Later, he developed warts, and persistent fungal nail infection. Based on recurrent staphylococcal infection, severe eczema, eosinophilia (eosinophil count 2.56) and increased serum IgE (IgE level 18030 in the first visit), the patient was diagnosed with autosomal recessive hyper-IgE syndrome (AR-HIES). His immunological work up is shown in (Table1). The patient was kept on prophylactic itraconazole, sulfamethoxazole and monthly intravenous

immunoglobulin (IVIG). Despite prophylactic treatment, he developed recurrent pneumonias and a severe skin infection which required frequent intravenous antibiotics.

At the age of 12 years the patient developed dysphagia and an endoscopy confirmed that he had herpes simplex esophagitis.

Later at the age of 20 years, he presented a nasopharyngeal mass and an unsteady gait, in addition to a picture of cholangitis. Investigations showed the presence of anemia, leukocytosis, thrombocytosis, raised liver enzymes, direct hyperbilirubinemia, and deranged clotting factors. Magnetic resonance imaging (MRI) revealed irregular shaped soft tissue mass in the right lateral wall of the nasopharynx with obliteration of the fossa of Rosen-Muller and bilateral enlarged cervical and right supraclavicular lymph nodes, in addition to mild cortical cerebellar atrophy (Supplemental Figure 2).

The histopathology of the nasopharyngeal mass showed EBV-related diffuse large B-cell lymphoma (DLBCL) (Supplemental Figure 3).

Additionally, a CT scan of the chest and abdomen showed a suspicious lesion in the liver with a dilated common bile duct (CBD) and enlarged lymph nodes in the axilla, mediastinum, abdomen, and pelvis. Magnetic resonance cholangiopancreatography (MRCP) was done and revealed a dilated CBD with intrahepatic biliary radicles consistent with inflammatory process and/or malignant cholangiocarcinoma.

Therefore, endoscopic retrograde cholangiopancreatography (ERCP) was performed. It confirmed the diagnosis of secondary sclerosing cholangitis with no malignant cells, and a sphincterotomy was performed.

For DLBCL, the patient was given one cycle of rituximab (anti-CD20), cyclophosphamide, and prednisone (R-CHOP chemotherapy). However, after one week, he was admitted to the intensive care unit with febrile neutropenia and septic shock. Cultures revealed pseudomonas and MRSA bacteremia, in addition to herpes simplex lesions involving the scrotum and tongue. He also had left eye endophthalmitis. Even after intensive treatment the patient developed loss of his vision in his left eye and bulbar palsy.

The repeated Pan-CT scan revealed marked regression of the right nasopharyngeal mass and draining lymph nodes. However, because of the significant morbidity related to treatment, the family decided for a supportive palliative care only. The patient passed away at the age of 21 years.

Patient P2:

The second patient was a brother of patient1. He was following in an immunology clinic since the age of five years with recurrent chest infections, persistent eczema, repeated mucocutaneous herpetic infections, disseminated warts and fungal infection of the nails. The patient was clinically diagnosed with AR-HIES. He had blood eosinophilia (eosinophil count 2.65) and increased serum IgE (IgE level 11830). His immunological workup is also shown in (Table 1). Prophylactic itraconazole and sulfamethoxazole were used. The patient had multiple admissions with sepsis, chest infections, skin infections including extensive perianal herpetic and MRSA lesions, in addition to sclerosing cholangitis.

At the age of 8 years, he presented with abdominal pain. An abdominal ultrasound and a CT scan revealed a right adrenal mass. A core biopsy confirmed the mass to be EBV-related adrenal leiomyoma. No malignancy was seen. Subsequently an adrenalectomy was performed which proved the diagnosis of an EBV associated benign smooth muscle tumor of the adrenal gland (Supplemental Figure 4).

At the age of 16 years, patient presented with numbness in the lower limbs and left foot drop which progressed over 2 months. Neurological examination showed upper motor neuron signs in the left side of body. MRIs of the brain and spine were consistent with demyelinating process (Figure 5).

CSF analysis showed a high lymphocyte count, high protein and normal glucose level. Cultures for bacteria, mycobacteria and fungi were negative, as were PCR for herpes simplex, EBV, varicella-zoster virus and cytomegalovirus. The patient was empirically started on methylprednisolone, antibiotics and IV-immunoglobulin for presumptive diagnosis of para-infectious encephalitis. Patient showed no clinical improvement with the management. We proceeded with a brain biopsy from right frontal lesions. It showed an infiltrating inflammatory process (Figure 6). Also, creutzfeldt cells were noted which can be seen in the demyelinating processes (although nonspecific). Immunohistochemistry confirmed the diagnosis of progressive multifocal leukoencephalopathy.

The patient's neurological status progressively deteriorated even with treatment, Therefore, the family decided for supportive palliative care only. A few weeks later the patient passed away.

Patient P3:

Patient 3 is a cousin of the first two patients. She presented in infancy and was suffering from persistent eczema, food allergy to cow milk, soya and egg, recurrent otitis media and chest infection since 9 months of age. She had failure to thrive and difficulty swallowing. She had blood eosinophilia (1.98/micl) and a high IgE level (116400). Her immunological workup is also shown in (Supplemental Table 1). The patient was started on prophylactic antibiotics and antifungals. Mutation analysis confirmed mutations in the DOCK8 gene. Confirmed mutation along with the personal and family history was the reason behind the transplantation decision. . Therefore, she underwent HSCT at 3 years of age from her HLA full matched sister. Post transplantation, donor cells engraftment using STR showed 100 % myeloid and lymphoid engraftment, in addition to total control of her infections and complete resolution of her eczema.

Methods:

The CBCD and eosinophil counts were performed by a completely automated analyzer. Serum immunoglobulins were performed by enzyme-linked immunosorbent assays (ELISA), the lymphocyte subsets by flow cytometry and the lymphocyte proliferation Panel for Mitogens and Antigens by an in vitro lymphocyte blastogenesis assay.

Genetic Analysis:

The DNA of participating patients was screened by a PCR for STAT3, TYK2 and DOCK8 mutations. There were no mutations in the STAT3 or TYK2 genes. The DOCK8 PCR product was analyzed by sequencing in both the forward and reverse directions. Then, we compared previously reported DOCK 8 mutations and our patients' mutations. DNA from lymphocytes from these individuals was used to amplify exon 35 and associated flanking sequences of the DOCK8 gene. Then sequence analysis was identified and shown in Fig 7. The DOCK8 protein expression was also assessed.

Results:

Sequence analysis identified two copies of missense mutation, c.4346C>T, in the coding region of the DOCK8 gene in all patients. This sequence variant, c.4346C>T, predicts an amino acid change of serine (S) to leucine (L) at codon 1449 of the DOCK8 protein (p.S1449L). This sequence variant is of unknown significance as it has not been reported, and is predicted to be benign and tolerated. The patients also carried mutation in a different splice site at position 5 after the end of exon 45 and the effect of the splice site mutation has not been reported previously. However, from this mutation no DOCK8 protein was detected by the western blot. Thus absence of the DOCK8 expression is significant and apparently pathogenic. Results are shown in Table2 and figure 7.

Discussion:

The most common reported manifestations of AR-HIES are severe eczema and recurrent infections including viral, bacterial and fungal. Some complications are rare and severe complications are reported only sporadically in the literature. Other complications like EBV-related adrenal leiomyoma are previously reported in patients with acquired immunodeficiency syndrome (HIV/AIDS) (12-18). In our family series, one of our DOCK8 deficient patients had developed EBV-induced adrenal leiomyoma as a rare complication of DOCK8 mutation. We have previously reported from our center EBV-related adrenal leiomyoma in a girl affected by DOCK8 mutation indicating the severity of this mutation in this family (7).

Another complication in this family which also points to the severity of the disease is the Progressive Multifocal Leukoencephalopathy (PML) in patient 2 at the age of 16 years. PML is a fatal demyelinating disease which can be caused by a slow virus infection like polyomavirus (JCV). It has been reported in immunocompromised patient like lymphoma patients and HIV patients as well as in two DOCK8 patients previously and one STAT3 patient (6, 19). Pathological confirmation of PML (on brain biopsy or autopsy) requires demonstration of the triad of demyelination, enlarged hyperchromatic oligodendrocyte nuclei and enlarged astrocytes with bizarre hyperchromatic nuclei. In our patient, the brain biopsy confirmed the presence of the JCV virus as the underlying cause of the demyelinating process. The development of PML due to JCV in our patient indicates the necessity to reconstitute the immune system in DOCK8 patients early enough especially in those with positive family history of severe diseases as we did with patient 3.

The risk of lymphoma in patients with AR-HIES syndrome may be as high as 259-fold over the general population (20-22). Although the exact etiology of malignant transformation in DOCK8-deficient patients is not clearly understood, in addition to the loss of T cell surveillance of tumor cells, it is suggested that DOCK8 protein may play a direct tumor suppressor role, which is lost in these individuals (23). This theory is supported by the observation of decreased expression of the DOCK8 protein in cell lines of patients with lung cancer and soft tissue sarcomas (24, 25)

HSCT is the only curative therapy available for DOCK8 deficiency (26). An increased risk of malignancy in DOCK8 patients may still play a tumor suppression role in non-hematopoietic tissues even after HSCT(27).

Diagnosing the cousin and giving her a transplant early enough from the family matched donor proved to be successful with full engraftment and completely free of disease manifestations. Many groups have reported sporadically successful transplantation for DOCK8 deficient patients.(28-37)

Many mutations could be involved in DOCK8 deficiency. Some of these novel mutations were reported from our center(8). This is mostly due to consanguineous marriages in our community, reaching up to 60%. Similar patterns have been reported in neighboring countries like Kuwait (38). However, in this reported family, all affected patients carried a novel splice site mutation at position 5 after the end of exon 45 resulting in a stop codon and leading to a truncated protein. This mutation has not been reported previously in the literature.

Conclusion:-

Hereby we report one family affected clinically with AR-HIES syndrome due to DOCK8 mutation which is pathogenic and novel. The clinical presentation, course and complication of this mutation proved to be severe and fatal if not treated early with stem cell transplantation. On the other hand HSCT in early childhood had led to full engraftment, excellent outcome and complete cure. Thus, we recommend early detection of this otherwise fatal disease and prompt management with stem cell transplantation.

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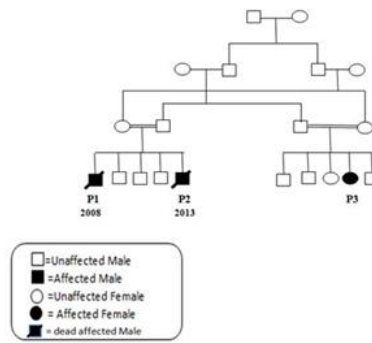
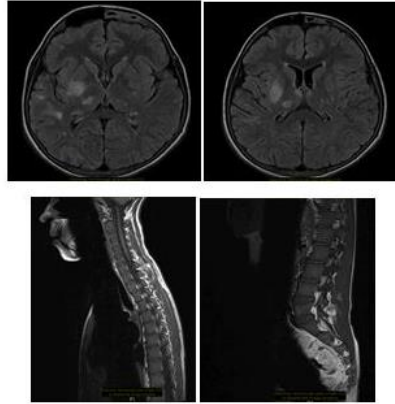


Figure 1. Family pedigree

Figure 1, Family Pedigree



MRI brain and whole spine
Multiple lesions in white matter

Figure 5. MRI brain and whole spine showing multiple lesions in white matter.

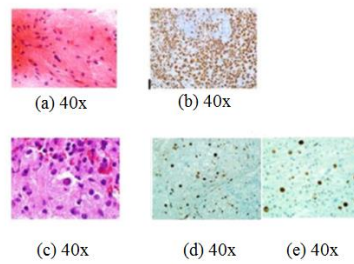


Figure 6. (a) Very reactive astrocytosis.(b) CD68 highlights the histiocytes infiltrate. (c) High power of infected oligodendroglial cells with inclusion and displaced chromatin to periphery. (d) Infected cells positive for Ki-67. (e) Infected cells positive for P53.

Figure 6. (a) Very reactive astrocytosis.(b) CD68 highlights the histiocytes infiltrate. (c) High power of infected oligodendroglial cells with inclusion and displaced chromatin to periphery. (d) Infected cells positive for Ki-67. (e) Infected cells positive for P53.

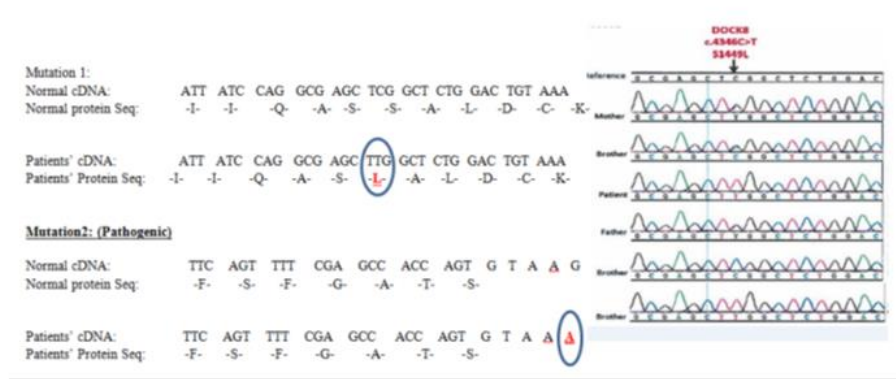


Figure 7 Mutation analysis showing novel mutations, with protein changes.

Patient	Eosino phil /micl	IgE IU/l	Ig G g/l	IgA g/l	Ig M g/l	CD 3 /m m	CD 4 /m m	CD 8 /m m	CD 19 /m m	CD16/ 56 /mm	Lymphoc ytes Proliferat ion Response To PHA (CPM)	Outco me	Clinical Presentation
P1 Last visit	0.65	2235	2.7	0.3 6	<0. 18	42 1	14 3	23 7	<1	199		Died Sepsis	Recurrent sinuopulmonar y infections, eczema, recurrent staphylococcal infection, warts, failure to thrive, sclerosing cholangitis, chronic inflammation of skin like impetigo picture, herpes simplex esophagitis, diffuse large B cell lymphoma.
P1 At presentati on	2.56	1803 0	12. 9	1.8	0.2 9	13 46	53 8	58 8	734	367	14175 (17%)		
P2 Last visit	1.44	1272	18	<0. 25	0.4 2	88 2	33 3	43 3	259	187	44928 (4%)	Died Sepsis	Recurrent sinuopulmonar y infections , eczema, recurrent mucocutaneo us hepatic infections, warts, Failure to thrive, bronchiectasis, sclerosing cholangitis, warts, EBV infection, adrenal gland leiomyoma, progressive multifocal leukoencephal opathy (PML)
P2 At presentati on	1.90	1183 0	11	<0. 25	0.2 9	14 92	44 3	72 3	653	140	7317 (29%)		
P3 Pre- transplant ation	1.98	1164 00	10. 7	0.7 0	0.5 9	24 40	52	10	992	11	18630 (8%)	Alive Post BMT	Recurrent sinuopulmonar y infections, eczema, failure to thrive
Post transplant ation	0.70	-	11. 9	4.6 0	0.2 2	33 60	16 19	15 67	132 4	255	126290 (86%)		

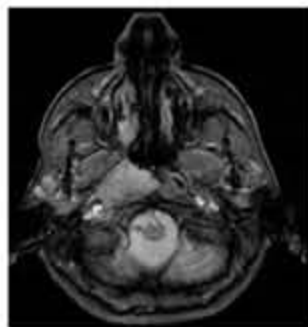
Normal reference values: [CD3(1-11 m:3,100-4,800, 1-2 years: 2,200-4,100, 3-5 years: 1,600-2,700, 6-13 years:1,700-1,900 per mm³), CD4(1-11 m: 2,200-3,300, 1-2 years:1,400-2,800, 3-5 years: 1,00-1,700, 6-13 years:800-1,700 per mm³), CD8 (1-11m: 1,100-1,700, 1-2 years: 800-1,800, 3-5 years:600-1,000, 6-13 years: 700-1,000 per mm³), CD19 (1-11 m: 1,100-1,900, 1-2 years: 700-1,600, 3-5 years: 400-800, 6-13 years:400-800 per mm³), CD16CD56(1-11m:300-700, 1-2 years:200-600, 3-5 years:200-400, 6-13

years:200-400 per mm³)) pediatric allergy and immunology 1998; 9:44-48], IgG 2.5-9.1 g/L, IgA 0.2-1.2 g/L, IgM 0.2-1.5 g/L, IgE 1.6-30 u/ml and PHA 94,935-171,149 CPM

Table 1 Clinical and Laboratory characterization

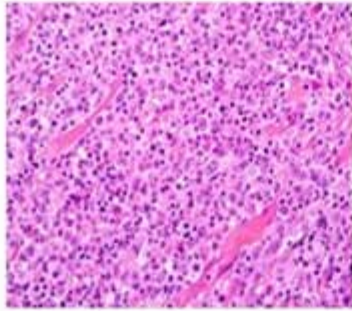
Table 2. Mutation analysis showing novel mutations, with protein changes.

Gene affected	DNA change	Protein Change	Predicted effect on gene/gene function
DOCK8	Mutation1: Homozygeous mutation for c. 4346 C>T from variant one Mutation 2: Homozygous mutation for c.4626+5 G>A from variant one	Mutation1: S1449L Mutation2 : Splice site Mutation at position 5 after at the of exon 45	Mutation 1: A change from a conserved hydrophilic polar (S: serine) to hydrophobic nonpolar (L:Leucine). Expected to be not a pathogenic change as predicted by Polyphen-2. However, a normal protein is expected to be expressed from the normal allele. Mutation 2 : Conserved splice site mutation. The effect of the splice site mutation has not been determined. However, no DOCK 8 protein was detected by western blot. The absence of the DOCK* expression is significant and is expected to lead to be pathogenic of HIES.

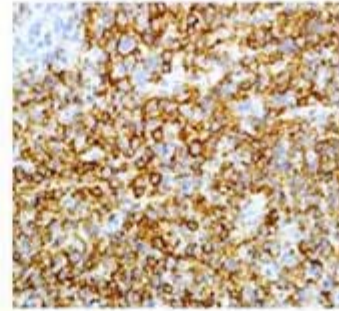


MRI of Head/Neck
with nasopharyngeal mass

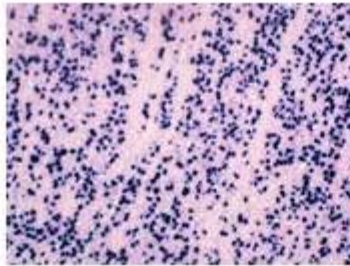
Figure S 2



(a) 20 x

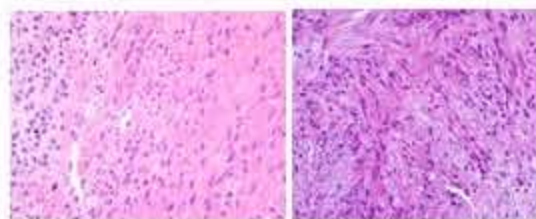


(b) 40 x



(c) 40 x

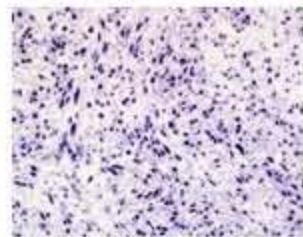
Figure S 3 (DLBCL) (a) large neoplastic lymphoid cells diffusely infiltrating among myofibers. (b) CD20 Immunostaining highlights the large B cells. (c) EBV in situ hybridization positive.



(a) 40x



(b) 60x



(c) 40x

Figure S 4

Patient	Eosinophil/ micl	IgE IU/L	IgG g/l	IgA g/l	IgM g/l	CD3 /mm ³	CD4 /mm ³	CD8 /mm ³	CD19 /mm ³	CD16/56 /mm ³	Lymphocytes Proliferation Response for PHA (CPM)	Outcome	Clinical Presentation
P1 Last visit	0.65	2235	2.7	0.36	<0.18	421	143	237	<1	199	-	Died, sepsis	Recurrent sinopulmonary infections, eczema, recurrent staphylococcal infection, warts, failure to thrive, sclerosing cholangitis, chronic inflammation of skin like impetigo picture, herpes simplex esophagitis, diffuse large-B cell lymphoma
at presentation	2.56	18030	12.9	1.8	0.29	1346	538	588	734	367	14175 (17%)		
P2 Last visit	1.44	1272	18	<0.25	0.42	882	333	433	259	187	44928 (4%)	Died, sepsis	Recurrent sinopulmonary infections, eczema, recurrent mucocutaneous herpetic infections, warts, Failure to thrive, bronchiectasis, sclerosing cholangitis, warts, EBV infection, adrenal gland leiomyoma, progressive multifocal leukoencephalopathy (PML)
at presentation	1.90	11830	11	<0.25	0.29	1492	443	723	653	140	7317 (29%)		
P3 Pre- Transplantation	1.98	116400	10.7	0.70	0.59	2440	52	10	992	11	18630 (8%)	Alive, Post- BMT	Recurrent sinopulmonary infections, eczema, failure to thrive
Post- Transplantation	0.70	-	11.9	4.60	0.22	3360	1619	1567	1324	255	126290 (86%)		

Normal reference values: [CD3(1-11 m: 3,100–4,800, 1-2 years: 2,200–4,100, 3-5 years: 1,600–2,700, 6-13 years: 1,700–1,900 per mm³), CD4(1-11 m: 2,200–3,300, 1-2 years: 1,400–2,800, 3-5 years: 1,000–1,700, 6-13 years: 800–1,700 per mm³), CD8 (1-11 m: 1,100–1,700, 1-2 years: 800–1,800, 3-5 years: 600–1,000, 6-13 years: 700–1,000 per mm³), CD19 (1-11 m: 1,100–1,900, 1-2 years: 700–1,600, 3-5 years: 400–800, 6-13 years: 400–800 per mm³), CD16+ 56+ (1-11 m: 300–700, 1-2 years: 200–600, 3-5 years: 200–400, 6-13 years: 200–400 per mm³) pediatric allergy and immunology 1,998; 9:44–48]. IgG 2.5–9.1 g/L, IgA 0.2–1.2 g/L, IgM 0.2–1.5 g/L, IgE 1.6–30 u/ml and PHA 94,935–171,149 CPM