4 CASES FROM SUSPECTED CHRONIC GRANULOMATOUS DISEASE IN THE RESPIRATORY UNIT IN VIETNAM NATIONAL CHILDREN'S HOSPITAL

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Chronic granulomatous disease (CGD) is an inherited primary immunodeficiency disease which is one kind of the phagocytic dysfunction. It is accounted for 1 : 200000 live births in the United States. The mechanism of CGD is mutation in any structural molecules of Nicotinamid Adenine Dinucleotide Phosphate (NADPH) oxidase. Therefore, CGD increases the body's susceptibility to infections caused by bacteria and fungi with granulomas formed at the sites of infection or inflammation. We report 4 cases diagnosed as suspected CGD in patients suffering from persistent pneumonia in the respiratory unit in Vietnam National Children's Hospital to recommend colleagues not to mis-diagnose CGD in recurrent or persistent pneumonia.

Keywords: chronic granulomatous disease, persistent pneumonia.

I. INTRODUCTION

CGD is a rare disease which can be mis-diagnosed in recurrent or persistent pneumonia. One of the reasons is the lack of DHR test (Dihydrorhodamine flow cytometry based test) in the laboratory. However, Vietnam Central Children's hospital have had DHR test for screening of suspected patients suffering from CGD for approximately 1 year. Therefore, we want to report 4 case studies diagnosed with persistent pneumonia in patients suffering from suspected CGD, to recommend all colleagues not to mis-diagnose CGD in the clinical practice. We collected clinical and sub-clinical data from patient charts, then followed up patients after discharged.

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Case 1

A 4 month-old boy who admitted to hospital because of cough and wheezing. The patient was treated for pneumonia from 38 days to 4 months old without improvement. Pre-history: he was the third child with a normal birth weight of 3.8 kg. His older sister and older brother are healthy.

On admission, the child was suffering from respiratory distress grade 2 with wet rales in both lungs with hepatomegaly, splenomegaly and lymphadenomegaly. Full blood count (FBC) showed increased white blood cells (WBC), especially neutrophils. Dihydrorodamine (DHR) flow cytometry based test showed phagocytic dysfunction in quality. Chest X-ray and Multi-slice computer tomography (MSCT) showed pneumonia, lymph node in mediastinum, mild pleural effusion. Bacteria and fungi count were negative. The patient was treated by antibiotics and anti-fungal drugs, then discharged home with oxygen support.

Table 1. Peripheral blood test

	5th Jan 2020	10th Jan 2020	16th Jan 2020	11th Feb 2020	21st Feb 2020	28th Feb 2020	9th Mar 2020	23rd Mar 2020	31st Mar 2020
WBC (G/l)	20.73	24.03	28.77	21.63	25.65	25.64	31.55	30.88	35.16
Neu(%)	43.5	52.9	54.4	47.3	55.7	54	57.6	62.3	62.4
Lym(%)	33	28	25.2	39.7	34.4	30	30.9	25.9	26.7
Eosin(%)	6.5	4.5	3.5	1	0.3	1.7	1.4	1.7	0.9
Baso (%)	0.1	0.2	0.1	0.1	0.2	0.4	0.3	0.2	0.2
Hb (g/l)	113	109	94	81	94	97	104	98	96
PLT (G/l)	464	446	442	465	259	364	512	491	646
CRP (mg/l)	56.26							57.33	33.34
Pro-calcitonin (ng/ml)		0.56		0.53	0.48	0.28			

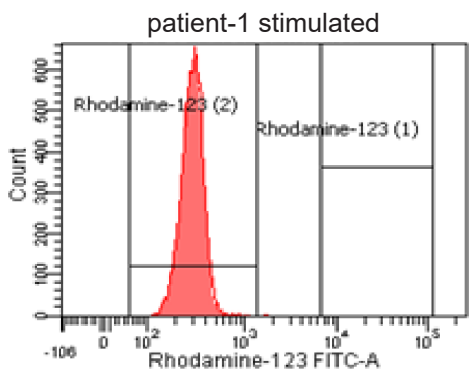

Image 1. DHR test of case-1 showed Neutrophil dysfunction

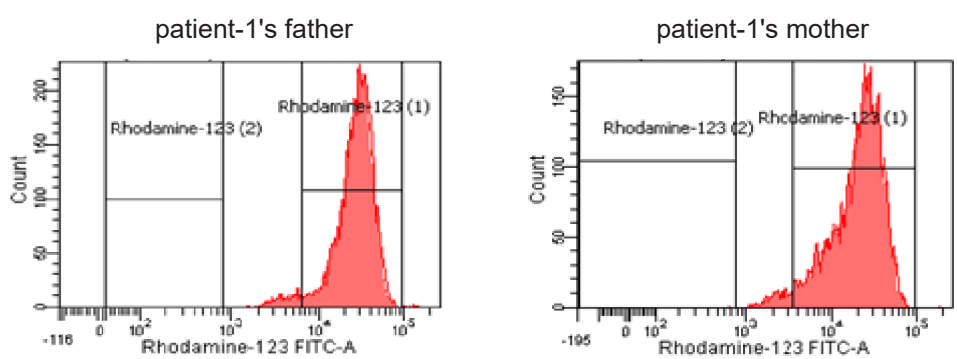

Image 2. DHR test of case 1's parents showed normal Neutrophil function

Table 2. Lymphocytes subset in peripheral blood

Cells	Total (cells/μL)
CD4 (TCD4)	2609.8
CD8 (TCD8)	2189.08
CD3 (Lympho T)	4940.15
CD19 (Lympho B)	1479.6
CD56 (NK cell)	158.89

Table 3. Immunoglobulin in Serum

Immunoglobulin	**Concentration (g/L)**
IgA	0.47
IgG	11.79
IgM	1.15
IgE	302.5

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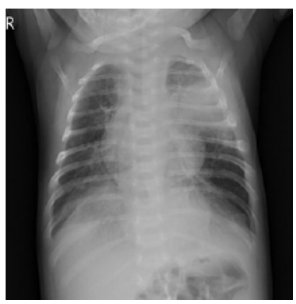
11th January 2020



30th March 2020

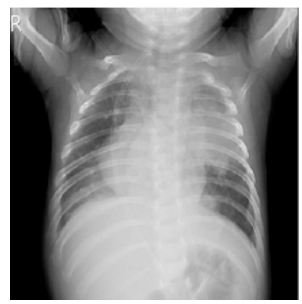
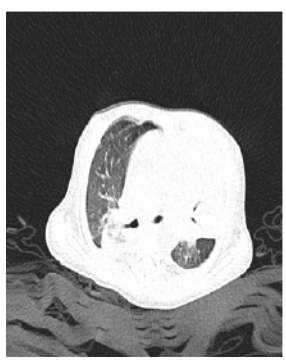


Image 3. Chest X-ray showed opacity in the right lung without improvement

MSCT lung

10th February 2020



3rd April 2020

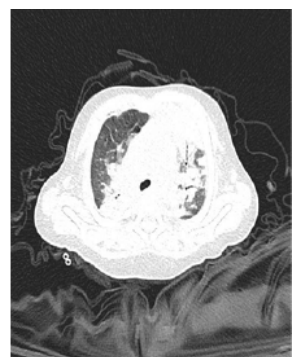


Image 4. MSCT lung show pneumonia, does not suggest congenital pulmonary malformation

The pathogens of pneumonia were negative.

The patient's bacterial culture in the nasopharyngeal and bronchoalveolar lavage (BAL) fluid was negative; Polymerase Chain Reactive (PCR) for 7 bacteria in the respiratory tract was negative; PCR tuberculosis was negative 3 times, Microscopic Observation Drug Susceptibility liquid culture (MODS) tuberculosis was negative 3 times; Mycobacterium tuberculosis (MTB) resistant to Rifampicin (RMP) expert was negative, Quantiferon was negative.

PCR Pneumocystis pneumonia (PCP) was negative, fungal culture was negative, test for fungal antigen was negative.

Influenza type A, B; RSV; Adenovirus, Cytomegalovirus (CMV) were all negative.

Human Immunodeficiency Virus (HIV) Enzyme-Linked Immunosorbent Assay (ELISA) was negative.

Bronchoscopy showed airway inflammation.

Case 2

A 4 month-old boy, admitted to hospital because of cough and fever. The child was treated for pneumonia from age 2 months old to 4 months old without improvement. Pre-history, he was the first child, normal birth weight.

On examination, he was febrile with wet rale in the right lung, no respiratory distress. FBC showed increased WBC, especially neutrophils. DHR flow cytometry based test showed phagocytic dysfunction in quality. Chest X-ray and MSCT showed pneumonia, mild pleural effusion. The pathogen of bacteria and fungi were negative. The patient was then treated with antibiotics and anti-fungal drugs and is still an in-patient.

Table 4. Peripheral blood test

	19th March 2020	31st March 2020	6th April 2020	9th April 2020	10th Apr il 2020
WBC (G/l)	47.62	26.14	24.61	20.66	28.73
neu (%)	40.8	43.8	48.7	82.5	89.3
Lympho (%)	38.2	42.6	35.4	14.6	7.4
Hb (g/dl)	109	111	109	106	109
PLT (G/l)	685	516	563	507	308

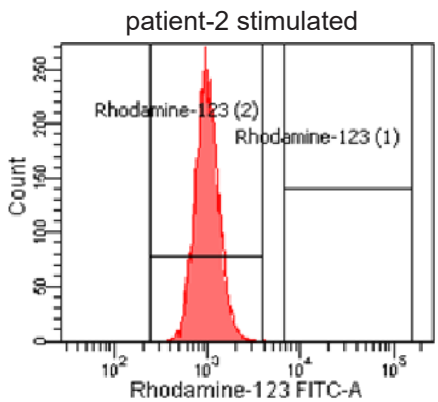
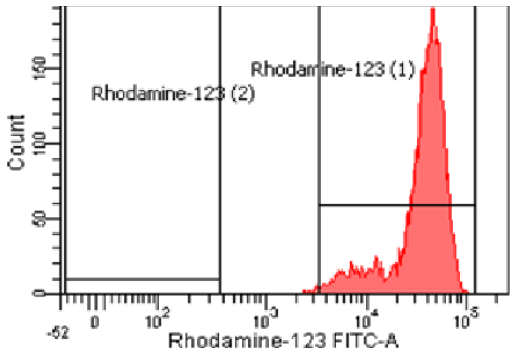


Image 5: DHR test of case 2 showed Neutrophil dysfunction

patient-2's father stimulated



patient-2's mother stimulated

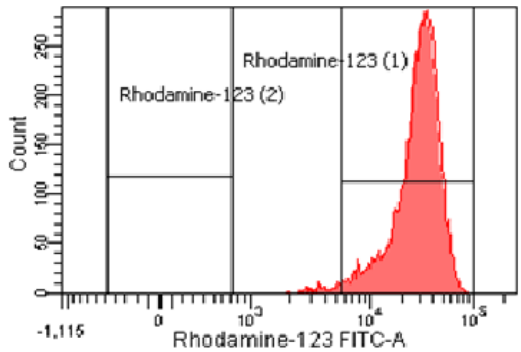


Image 6. DHR test of case-2's patients showed normal neotrophil function

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9th April 2020

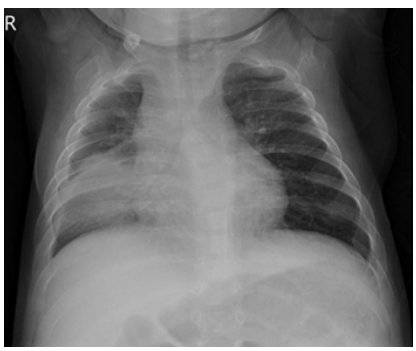

Image 7

30th March 2020

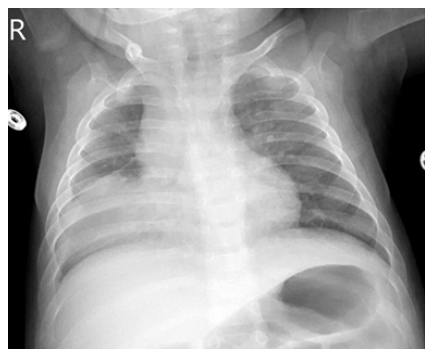

Image 8

19th March 2020

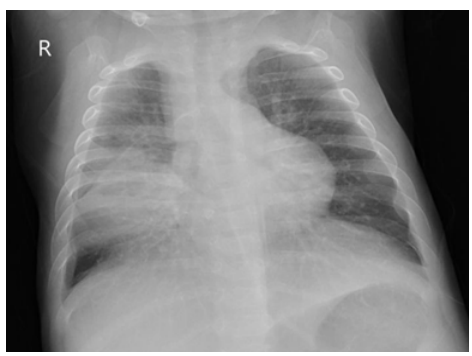

Image 9

Image 7,8,9. Chest X-ray showed opacity in the right lung without improvement

The pathogens of pneumonia were negative.

The patient's bacterial culture in the nasopharyngeal fluid was negative, PCR for 7 bacteria in the respiratory tract was negative; PCR tuberculosis in the gastric fluid was negative 3 times, MODS tuberculosis in the gastric fluid was negative 3 times; Acid Fast Bacillus (AFB) tuberculosis in the gastric fluid was negative 3 times; MTB resistant to RMP expert was negative.

PCR PCP was negative, fungal culture were negative.

Case 3

A 11 month-old boy, prehistory:

o First time: he was treated for pneumonia from age 19 days old to 7 weeks old.

o Second time: after being discharged home for 3 weeks, he was admitted to the hospital again because of pneumonia and treated for 4 weeks.

o Third time, patient was admitted and treated for pneumonia and Crohn's disease for 20 days.

o Fourth time, patient was admited and treated for pneumonia, respiratory distress/

Table 5. Peripheral blood test

	27th June 2019	10th July 2019	22nd July 2019	8th August 2019	23rd August 2019
WBC (G/l)	21.76	33.76	22.39	18.1	21.56
Neu (%)	70.3	70	67.2	45.3	52.1
Lym (%)	23.2	18	26.3	45.6	41.9
Hb (g/l)	87	97	98	109	113
PLT (G/l)	638	811	753	648	707

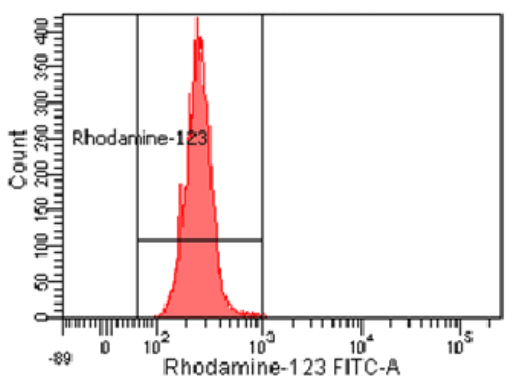

Image 10. DHR test of case-3 showed neutrophil dysfunction

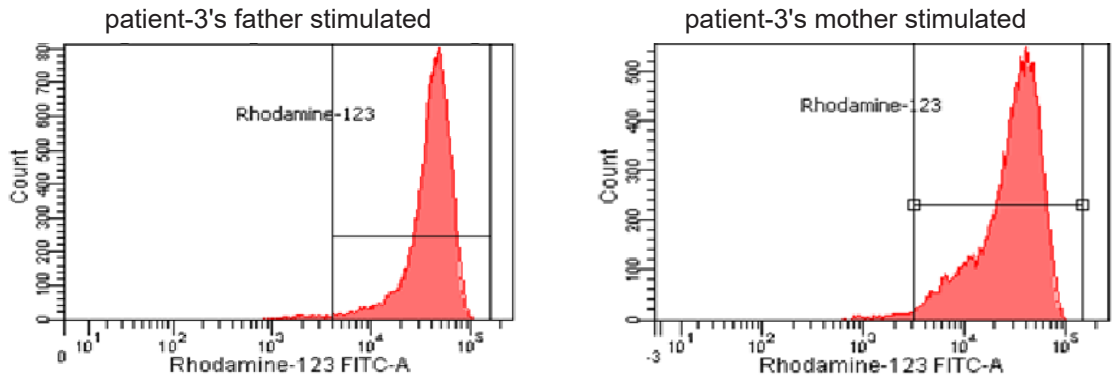

Image 11. DHR test of case-3 parents showed normal neutrophil function

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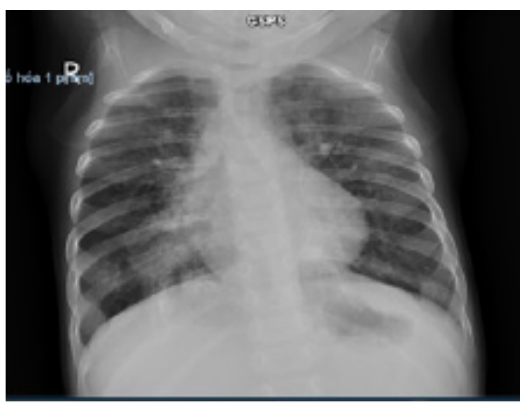


Image 12. Chest X-ray showed opacity in the right lung

Immuno test:IgA: 0.54 g/l, IgM: 1.34 g/l, IgG: 10.39 g/l, IgE: 6.1 g/l; CD3 cells:6244 cells/mm3, CD4 cells: 3278 cells/mm3, CD8 cells: 2798 cells/mm3.

HIV was negative by ELISA

Case 4:

A 2.5 year-old-boy, pre-history: recurrent pneumonia.

• First time: when he was 30 days of age, he was admitted and treated for pneumonia and acute diarrhea for 38 days.

• Second time: 1 month later, he was treated for pneumonia for 52 days.

• Third time: He was treated for pneumonia and hand-foot-mouth disease when he was 24 months old.

From 24 months old till now, he was treated for recurrent pneumonia 4 times at the hospital without improvement.

Table 6. Peripheral blood tests

	3rd July 2019	10th July 2019	25th July 2019	6th August 2019
WBC (G/l)	23.53	27.1	11.96	11.91
Neu (%)	12.4	18.76	7.99	5.76
Hb (g/l)	103	95	92	89
PLT (G/l)	595	664	631	759
CRP (mg/l)	47.89	118.3	119.27	138.58

DHR flow cytometry based test showed phagocytic dysfunction in quality

Table 7. Lymphocyte subset in peripheral blood

Cells	Cells/µL
CD4 (TCD4)	1487
CD8 (TCD8)	1507
CD3 cells (Lympho T)	3053

Table 8. Immunoglobulin in Serum

Immunoglobulin	Concentration (g/L)
IgA	0.86
IgG	8.75
IgM	1.57

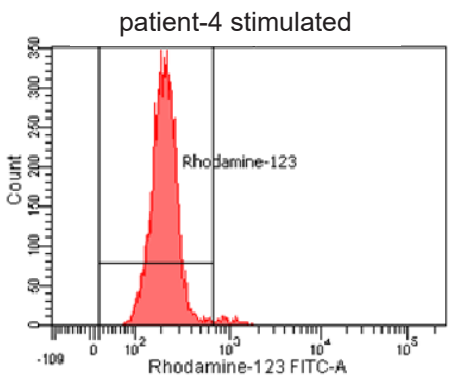
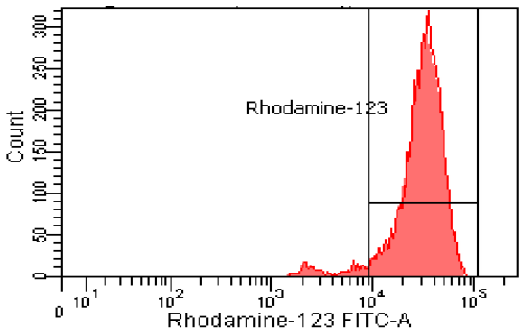


Image 13. DHR test showed neutrophil dysfunction

patient-4's father stimuated



patient-4's mother stimuated

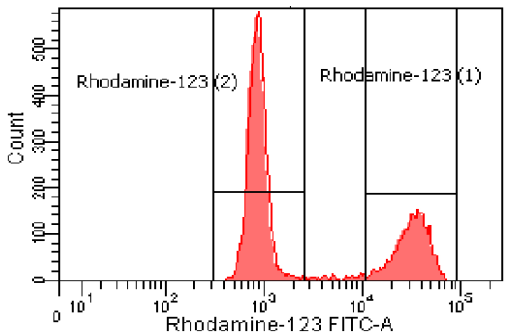


Image 14. DHR test of case-4's father showed normal neutrophil function, the mother's DHR test showed 2 populations

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16th February 2019

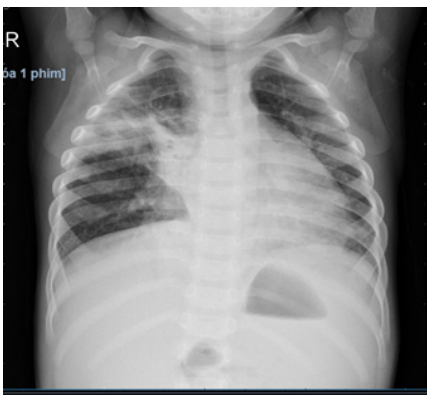

Image 15

3rd July 2019

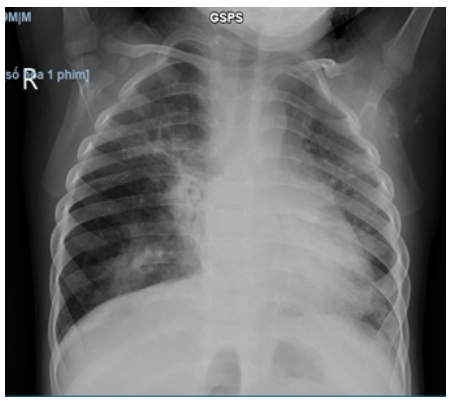

Image 16

26th August 2019

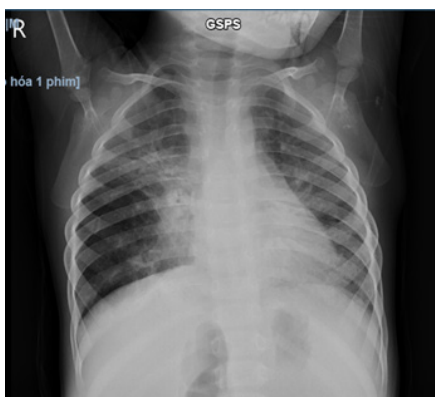

Image 17

II. DISCUSSION

All 4 patients were diagnosed with recurrent and persistent pneumonia. Most of them were diagnosed with pneumonia at an early age (patients were diagnosed with pneumonia when he was 19 days old, 30 days old, 38 days old). There was a slow clinical improvement despite testing to detect the pathogens that cause pneumonia such as bacterial, viral, fungal culture in nasopharyngeal, bronchoalveolar lavage (BAL) fluid, bronchoscopy and imaging diagnostic as well as suitable treatment. In addition, chest Xray and lung MSCT of 4 cases showed opacity of the lung without improvement. Their cell immunity was in normal range, while their immunoglobulins were slightly increased. Marciano BE at al. (2015) examined records of 268 patients at a single center over 4 decades to understand the impact of common severe infections in Chronic granulomatous disease (CGD) . Aspergillus incidence was estimated at 2.6 cases per 100 patient-years; Burkholderia, 1.06 per 100 patient-years; Nocardia, 0.81 per 100 patient-years; Serratia, 0.98 per 100 patient-years, and severe Staphylococcus infection, 1.44 per 100 patient-years. Lung infection occurred in 87% of patients.[4]

In addition, the increased WBC, especially neutrophils led us to the DHR (Dihydrorhodamine 123 test) which finally helped screened for CGD. The nonfluorescent rhodamine derivative, DHR, is taken up by phagocytes and oxidized to a green fluorescent compound byproducts of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase). The sensitivity and quantitative nature of this assay make it possible to differentiate oxidase-positive from oxidase-negative phagocyte subpopulations in CGD carriers and identify deficiencies in gp91phox and p47phox.[5] Abnormal DHR test results can be seen in other diseases included G6PD (glucose-6 phosphate dehydrogenase), myeloperoxidase deficiency and the syndrome of synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO).[6]

After the positive neutrophil-function testing, positive findings should be confirmed by genotyping. We are waiting for the result of genotyping of case 2 which is the first child of the family. Parents of the 3 other patients did not approve genotyping since the siblings are healthy and genotyping is an expensive test.

1 of 4 patients was misdiagnosed as Crohn's disease which is the differential diagnosis to CGD.[7]

In addition, one patient was diagnosed for CGD when he was 2.5 years old. He was treated for pneumonia twice including the first time of admission when he was 30 days old of age and the second time when he was 3 months old; afterward, he was healthy for 6 months. CD3, CD4, CD8 and IgA, IgG, IgM tested were in the normal range. WBC decreased from high to normal range due to treatment response which explained why the patient was diagnosed for CGD later than the 3 other patients. In this case, we try to exclude MTB which was the cause of pneumonia by doing a bronchoscopy, then test the BAL in our hospital and at the Lung Center Hospital. Both results were negative.

Successful hematopoietic cell transplantation (HCT) is a definitive cure for CGD.[8] Success increases and morbidity and mortality are reduced, early HCT becomes a desirable and appropriate choice for patients with CGD. The estimated HCT event-free survival rate for patients with CGD is > 80 percent; overall survival is approximately 90 percent, with improved the quality of life and transplant outcomes continues to improve.[9] However, in Viet Nam, HCT is very costly, from hundreds of millions to one or

two billion Vietnamese dong, and is only partially reimbursed by health insurance. The cost is too high for many families with patients suffering from immunodeficiency diseases to pursue extended treatment. Consequently, it remains the largest barrier for patients to approach root treatment.

III. CONCLUSION

Chronic granulomatous disease is a rare primary immunodeficiency disease, easily misdiagnosed. Patients are susceptible to bacterial, fungal, and tuberculosis infections, anemia, slow growth, and slow wound healing. Symptoms are evident in many systems including respiratory, digestive, urogenital, skin, eyes, and mouth.

As CGD can be shown in many organs and since physicians are not familiar with the DHR test, the diagnosis can be missed. Therefore, as presented in the aforementioned 4 cases study, we recommend to implement the DHR test in children with severe, recurrent and persistent infections to attain appropriate diagnosis and treatment plan.

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