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NEUTROPHIL, MONOCYTE AND LYMPHOCYTE LOCOMOTION IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS*

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Summary: Ertuğ H, Arman M, Yeğin O. (Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Turkey). Neutrophil, monocyte and lymphocyte locomotion in rheumatic fever and rheumatoid arthritis. Turk J Pediatr 32: 73-78, 1990.

Neutrophil, monocyte and lymphocyte chemotaxis was investigated in 19 patients with active rheumatic fever (10 with carditis), in 15 patients with rheumatoid arthritis and in 20 healthy, age-matched controls. Chemotaxis assays were repeated in the rheumatic fever patients on the fifth day of therapy and two weeks after remission. Neutrophil and monocyte chemotaxis was found to be significantly decreased in the rheumatoid arthritis patients when compared with the controls and rheumatic fever patients. In contrast, neutrophil chemotactic activity was significantly higher in the rheumatic fever patients when compared with the healthy controls. Monocyte and lymphocyte chemotaxis in patients with rheumatic fever was not significantly different when compared with the controls. Neutrophil, monocyte and lymphocyte locomotion was found to be significantly decreased on the fifth day of salicylic acid or prednisolone treatment. *Key words: rheumatoid arthritis, rheumatic fever, chemotaxis, neutrophil, monocyte, lymphocyte.*

Both rheumatoid arthritis (RA) and acute rheumatic fever (RF) are known as inflammatory connective tissue disorders. Various abnormalities in inflammatory response have been postulated in both diseases. Although the pathogenesis seems to be different in these two diseases, their exact pathogenetic mechanisms are still obscure[1,2].

Immune response and inflammatory reaction develop concomitantly, and the elimination of antigen can be achieved without a clinically obvious inflammatory reaction. But in certain conditions, such as high antigen load, abnormal location of antigen, and antigens difficult to eliminate, an uncontrolled and long lasting inflammatory reaction may develop.

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Since chemotaxis (accumulation of inflammatory cells at the site of the inflammatory reaction) is an important step in the inflammatory response, we investigated neutrophil, monocyte and lymphocyte chemotaxis in 19 RF patients. Studies were repeated at different stages of the disease. The same parameters were investigated in 15 adult RA patients and 20 healthy controls using the same method.

Material and Methods

Fifteen RA patients (identified according to the criteria of ARA)[3], 19 RF patients (diagnosed by the modified Jones criteria)[4] and 20 healthy, age-matched controls were studied. Ten of the 19 RF patients also had carditis. Chemotaxis studies were performed in all patients prior to treatment. Therapy was initiated which consisted of 80-100 mg/kg salicylic acid (for patients without carditis), and 1-2 mg/kg (maximum 60 mg) prednisolone (for patients with active carditis). Chemotaxis studies were repeated on the fifth day of therapy, and again 14 days after cessation of therapy when clinical and laboratory examinations (fever, sedimentation, CRP, etc) showed remission.

Chemotaxis method

Lymphocytes, neutrophils and monocytes were obtained from the peripheral blood by using the two-step method described by Boyum[5]. Chemotaxis was evaluated by the modified[6] Boyden chamber method[7] using millipore filters (a pore size of 3 microns for neutrophils and 5 microns for monocytes and lymphocytes). 2×10^6 neutrophils or monocytes were placed in the upper compartment and chemotaxis was measured by the leading front method, as described by Zigmond and Hirsch[8].

Lymphocyte locomotion was determined by the previously described method without prior overnight incubation[9].

Zymosan activated serum prepared from ten healthy controls was used as the standard chemotactic agent.

Since some patients were lost to follow-up, and a sufficient quantity of cells could not be obtained from some of the other patients, an equal number of patients could not be studied.

The Wilcoxon and Mann-Whitney –*U* tests were used for statistical comparisons.

Results

The results of the neutrophil chemotaxis studies are shown in Table I. Neutrophil chemotaxis in the RF patients before therapy was found to be significantly higher in comparison with the healthy controls ($p<0.05$), RA patients, and the RF patients on the fifth day of therapy ($p<0.01$). Neutrophil chemotaxis was found to

be significantly decreased in the RA patients in comparison with the healthy controls ($p<0.05$). Neutrophil chemotaxis in the RF patients was also found to be significantly depressed during the administration of therapy when compared with this group before given therapy ($p<0.05$) and the controls ($p<0.05$). After therapy, the values showed no significant difference when this group was compared with the controls and before they were administered therapy.

TABLE I: Results of Neutrophil Chemotaxis *(μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	71±14	10	N.T.		N.T.	
RF (TOTAL)	86±26	19	71±15	18	76±10	11
RF (PAIRED)	88±17	11	71±17	11	77±11	11
CONTROLS	83±14	20	N.T.		N.T.	

* Results were given as the mean ± 1 standard deviation.
RA=rheumatoid arthritis, RF=acute rheumatic fever, NT=not tested.

The results of monocyte chemotaxis studies are given in Table II. Monocyte chemotaxis was found to be significantly depressed ($p<0.05$) in the RA patients when compared with the controls and the RF patients prior to therapy. Monocyte chemotaxis in this group was found to be significantly depressed ($p<0.01$) on the fifth day of therapy when compared with before and after the administration of therapy and with the controls. There was no difference in monocyte chemotaxis of the RF patients before and after therapy and the controls.

TABLE II: Results of Monocyte Chemotaxis Studies (μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	42±9	9	N.T.		N.T.	
RF (TOTAL)	47±9	19	43±10	16	48±6	11
RF (PAIRED)	47±11	8	35±11	8	46±6	8
CONTROLS	51±8	20	N.T.		N.T.	

Results of lymphocyte locomotion are given in Table III. Although there was no significant difference found in lymphocyte locomotion in any of the groups, a difference was demonstrable in the paired-test of the RF patients before and after the administration of therapy. Lymphocyte locomotion was found to be significantly decreased in RF patients on the fifth day of therapy compared with this group before and after therapy ($p<0.05$).

TABLE III: Lymphocyte Locomotion Results (μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	38±3	8	N.T.		N.T.	
RF (TOTAL)	31±10	16	30±9	16	34±10	11
RF (PAIRED)	32±11	8	26±9	8	35±11	8
CONTROLS	35±8	20				

The RF patients were further divided into two subgroups according to the presence or absence of carditis. No statistically significant difference in chemotaxis was found in any of the cell types (Table IV).

TABLE IV: Results of Chemotaxis in Rheumatic Fever Patients With and Without Carditis (μm)

CELL TYPE	WITH CARDITIS	n	WITHOUT CARDITIS	n
NEUTROPHIL	89+12	10	83+17	9
MONOCYTE	46+9	10	48+10	9
LYMPHOCYTE	31+9	9	32+12	7

Discussion

Uncontrolled accumulation of inflammatory cells at any site may cause an excessive inflammatory reaction due to active molecules (lysosomal and, superoxide enzymes causing complement activation, etc) which are released from these cells. Conversely, the delayed arrival of inflammatory cells at the infection site may lead to a prolonged inflammatory reaction because the infective agent would have time to proliferate[10-12].

Neutrophil and monocyte chemotaxis was found to be decreased in RA patients in our study, confirming previous studies[13-16]. The obvious difference in neutrophil chemotactic response in RA and RF patients suggests that the effects of these two diseases on neutrophil chemotaxis is different, or different etiopathogenetic mechanisms are responsible for these two inflammatory diseases. The depression in neutrophil chemotaxis in RA patients was not very significant; 60 percent of their chemotaxis values were within one standard deviation of the healthy controls. Of the numerous studies reported on neutrophil chemotaxis in RA patients, we were able to find only one article describing this activity in RF patients (Naik et al)[17].

These investigators studied 30 patients with active rheumatic fever, 30 with active rheumatic heart disease, and 28 rheumatic fever patients in remission. In contrast to our results, neutrophil chemotaxis was found to be normal in all their groups. In our study neutrophil chemotaxis was found to be elevated during active RF. This controversy could be due to patient selection. We excluded all patients who had taken drugs 48 hours prior to the study.

We observed that neutrophil, monocyte and lymphocyte locomotion was significantly decreased on the fifth day of therapy. It has been reported that both salicylic acid and prednisolone can depress chemotaxis[18-21]. Since we could not find any difference between the RF patients with (given salicylic acid) and without carditis (given prednisolone) on the fifth day of therapy, both drugs seem to be equally effective in depressing chemotaxis (data not shown). There was no difference in neutrophil chemotaxis between the controls and the RF patients in remission (after therapy). This finding indicates that the increase in neutrophil chemotactic response is secondary to active RF disease, and not an inherited abnormality.

Since we found obvious differences in neutrophil chemotaxis between the RA patients, RF patients and healthy controls, using the same method and technical conditions, this observed depression in the RA patients and the increase in the RF patients are most likely not due to technical factors.

An increase in chemotaxis during active RF can be a part of the active inflammatory reaction but the exact mechanism remains to be elucidated. Both prednisolone and salicylic acid are used for the treatment of RA and RF because of their well-known antinflammatory effects. The therapy is followed up by drug monitoring and/or nonspecific laboratory tests such as CRP, and erythrocyte sedimentation rate. Since chemotaxis is an important phase of the inflammatory reaction, monitoring chemotactic activity during drug therapy and correlating these findings with the clinical response may provide valuable information for the researcher.

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NEUTROPHIL AND LYMPHOCYTE LOCOMOTION IN PATIENTS WITH SELECTIVE IgA DEFICIENCY*

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Summary: Coşkun Y, Sanal Ö, Ersoy F. (Immunology Unit, Hacettepe University Institute of Child Health, Ankara, Turkey). Neutrophil and lymphocyte locomotion in patients with selective IgA deficiency. Turk J Pediatr 32: 79-83, 1990.

Neutrophil chemotaxis was studied in 14 patients, twelve with selective and two with partial IgA deficiency. Twelve of the 14 patients were also evaluated for lymphocyte locomotion. Zymosan activated serum was used to stimulate cell migration, and Boyden chambers were used for both neutrophil and lymphocyte chemotaxis assays. Evaluation of the results of neutrophil and lymphocyte locomotion showed that chemotaxis to ZAS, random migration and chemotactic index values were not significantly different when comparing the patients with the controls. *Key words: selective IgA deficiency, lymphocyte locomotion, neutrophil locomotion.*

Neutrophil chemotaxis has been investigated in various diseases including immunodeficiencies and has been found to be impaired in some of these diseases[1-2]. Defective neutrophil locomotion has been described particularly in the Hyper–IgE, Chédiak–Higashi and lazy leukocyte syndromes[3-6]. Few studies are available regarding chemotaxis in other immunodeficiencies.

Although various aspects of neutrophil locomotion are known, studies on lymphocyte locomotion have only been carried out in recent years[7-11]. Reports have shown that defective lymphocyte locomotion may be present in some cases with primary immunodeficiencies[7,12-13]. In this study, we investigated neutrophil and lymphocyte chemotaxis in patients with selective IgA deficiency. As far as we know, studies on lymphocyte locomotion in selective IgA deficiency have not been reported in the literature.

Material and Methods

Neutrophil chemotaxis was studied in 14 patients, twelve with selective and two with partial IgA deficiency. Twelve of the 14 patients were also evaluated for

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lymphocyte locomotion. One of the patients with selective IgA deficiency had no symptoms, three patients suffered from hematologic disorders, and six patients, four with selective and two with partial IgA deficiency were diagnosed as having allergic diseases. The other four patients had chronic, recurrent infections. At the time of the study, the patients had no evidence of infection and neither had they been recipients of any medication. In addition, neutrophil chemotaxis was studied in 13 healthy controls and lymphocyte chemotaxis in 11 healthy controls whose ages ranged between 15-23 years.

Neutrophil locomotion was evaluated using the Boyden[14] filter method (three μ m pore size millipore filtres) with Zymosan activated serum (ZAS). Cell migration was measured using the leading front method[15].

Lymphocyte locomotion was evaluated by the method described by Gupta et al[16]. Briefly, after the isolation of mononuclear cells, monocytes were eliminated by carbonyl iron treatment. The number of contaminated monocytes, which was determined morphologically with peroxidase staining[17], was less than four percent. Chemotaxis was studied by using ZAS as a chemotactic factor and five μ m pore size millipore filters, and cell migration was measured by the leading front method[15].

Cell locomotion to ZAS stimulation / random migration (RM) ratios were expressed as a chemotactic index (CI) in both assays.

The Mann–Whitney *U* test was used for statistical analyses.

Results

Neutrophil chemotaxis to ZAS, random migration, and chemotactic index values in the patients were as follows: 66.7 ± 20.9 μm (42.7–111.0μm), 21.9 ± 4.7 μm (15.7–28 μm) and 3.0 ± 0.7 μm (2.2–4.5 μm), respectively. When compared with the control values, no significant difference was found (Fig. 1). Neutrophil chemotaxis, RM and CI values in controls were 60.9 ± 21.0 μm (117.1 ± 40.0 μm), 20.1 ± 4.6 μm (13.2 random 27.4 μm) and 3.0 ± 0.7 μm (2.3–4.8 μm), respectively. The mean values of lymphocyte chemotaxis to ZAS, random migration and chemotactic index in the patients were as follows: 43.9 ± 10.1 μm (29.4–60.0 μm), 16.1 ± 5.6 μm (9.7–28.0 μm) and 2.9 ± 0.7 μm (1.5–4.1 μm), respectively. Lymphocyte chemotaxis, RM and CI mean values of controls were as follows: 40.3 ± 10.2 μm (27.6–59.2 μm), 14.5 ± 3.0 μm (10.0–17.7 μm) and 2.8 ± 0.76 μm (1 6–3.4 μm), respectively. The chemotactic index was low (just below two standard deviations of the mean value) in only one patient, who had lower chemotaxis and higher random migration. In other patients, the values were not significantly different from the controls (Fig. 2).

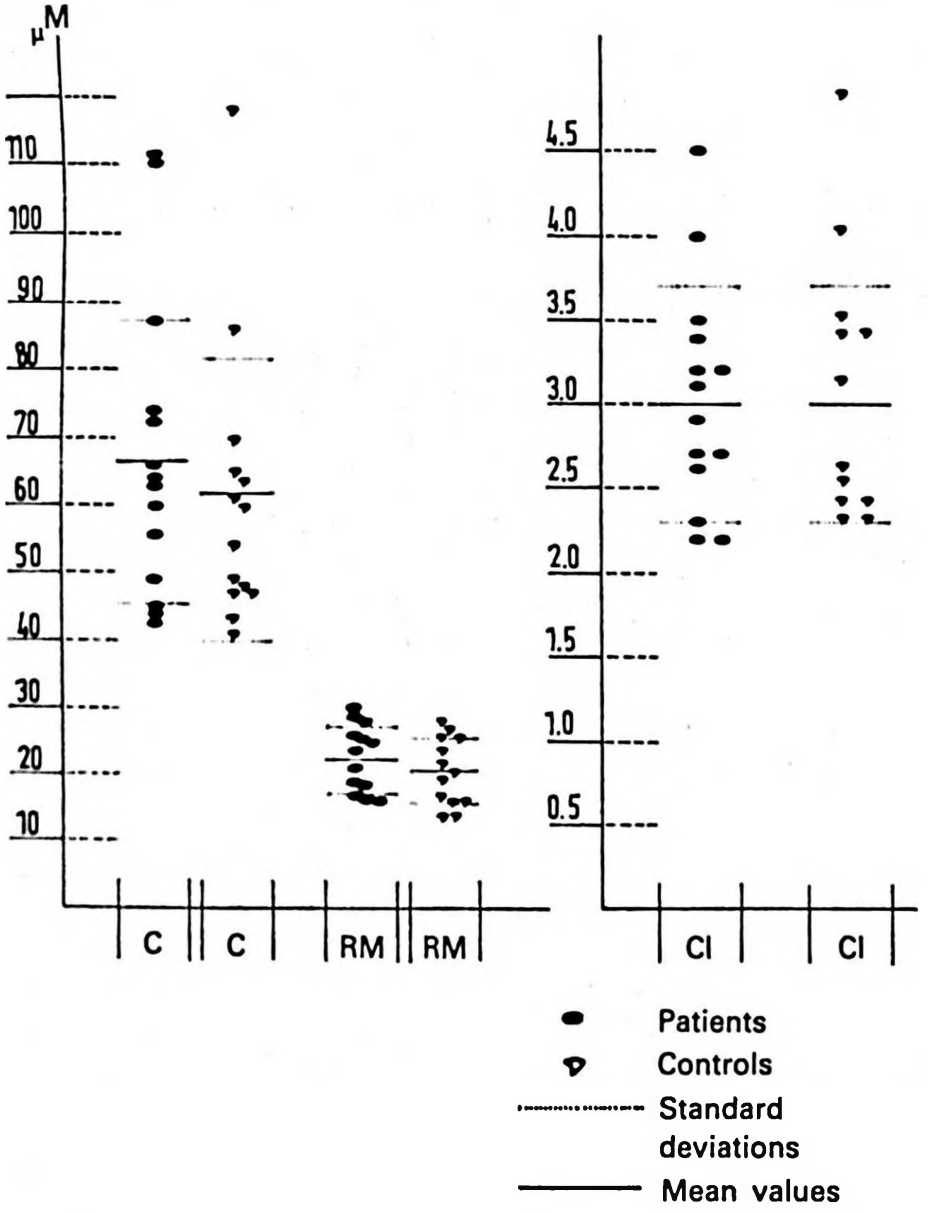

Fig. 1: Neutrophil chemotaxis (C), random migration (RM), and chemotactic index (CI) values in patients and controls.

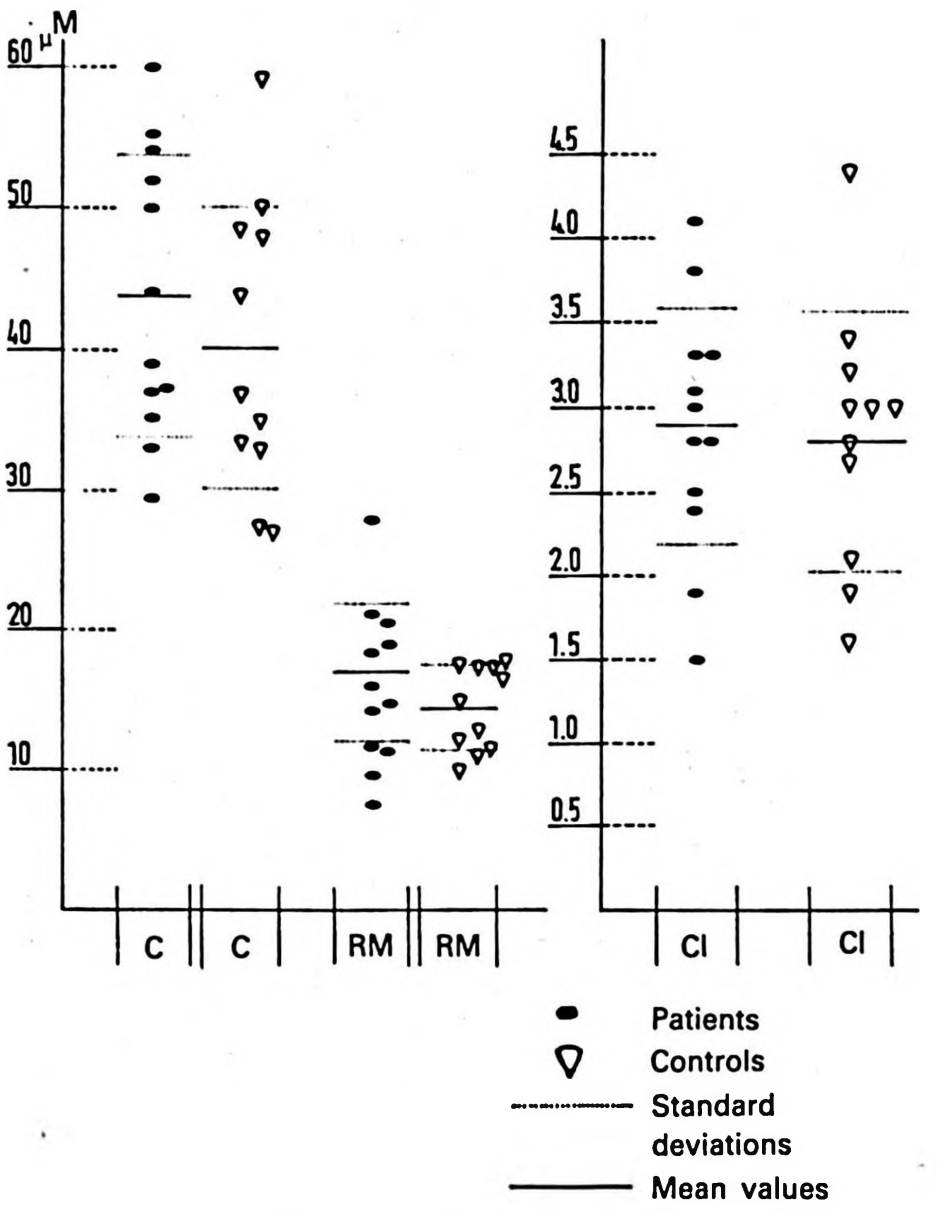

Fig. 2: Lymphocyte chemotaxis (C), random migration (RM), and chemotactic index (C) values in patients and controls.

Discussion

Chemotaxis, which is defined as the vectoral movement of cells governed by certain chemical substances, has an important role in the defense of microbial invasion. In addition to defects in other immune functions, the presence of a defective chemotactic response increases susceptibility to infection. It is known that in patients with the hyper–IgE, Chédiak–Higashi and lazy leukocyte syndromes, neutrophil locomotion is impaired[3-6]. It also has been demonstrated that there may be impaired neutrophil chemotaxis in patients with X–linked hypogammaglobulinemia, severe combined immunodeficiency, common–variable immunodeficiency, selective IgA deficiency and AIDS[18-22]. D'Amelio et al[18] found that nine out of ten patients with IgA deficiency had impaired leukocyte chemotaxis. But in our study no significant difference was observed in the chemotactic response, random migration or the chemotactic index when our patients were compared with the controls. It has been demonstrated that the chemotactic response reaches adult values after three years of age[23]. Therefore, the age difference between the patient and control groups had no effect on our results. D'Amelio et al[18] used the agarose method and ZAS as the chemotactic factor to evaluate chemotaxis. We also used ZAS to stimulate cell migration, but chemotaxis was evaluated using Boyden's[14] method. Even though we both used different techniques, it is unlikely that this caused a difference in our results. Impaired neutrophil motility may be caused either by a cellular defect or the presence of chemotactic inhibitors in serum. In this study, we could not find any cellular defect in our patients. However, additional studies are needed for the evaluation of chemotactic defects due to serum factors.

The results of lymphocyte chemotaxis in our patients also showed that there was no abnormality of lymphocyte locomotion in selective IgA deficiency. Van Epps et al[12] found impaired lymphocyte locomotion in some patients with primary immunodeficiencies by using C5a and casein as the chemotactic factors. In their study, lymphocyte chemotaxis was found to be defective in patients with common variable immunodeficiency, the hyper–IgE syndrome, and immunodeficiency with normal immunoglobulin concentrations, whereas it was found to be normal in patients with X–linked agammaglobulinemia and the hyper–IgM syndrome. Our findings do not exclude the fact that there might be, a part from this, a defect in T or B cell locomotion. Detailed studies regarding T and B cell locomotion are needed to clarify this subject.

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EXPERIENCE WITH PROPAFENONE FOR THE TREATMENT OF CARDIAC ARRHYTHMIAS IN CHILDREN*

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Summary: Çeliker A, Özer S, Özme S, Özkutlu S, Kahramanyol O. (Hacettepe University Institute of Child Health, Ankara, Turkey). Experience with propafenone for the treatment of cardiac arrhythmias in children. Turk J Pediatr 32: 85-92, 1990.

The aim of this prospective study was to evaluate the efficacy of propafenone in suppressing various arrhythmias in a group of children who were patients in the Pediatric Cardiology Unit of Hacettepe University Children's Hospital. Seven of the 13 children (53.8%) with ventricular ectopics, all three children with paroxysmal re-entry atrioventricular tachycardia, and two of the three children with paroxysmal atrial tachycardia were treated successfully with propafenone. The drug did not cause any side effects or arrhythmogenesis. The median dosage of propafenone at discharge was 300 mg/m^2/day (range 250-400 mg/m^2/day) every eight hours. Our findings confirm the efficacy and safety of propafenone for the treatment of childhood arrhythmias. *Key words: propafenone, arrhythmia, child.*

Propafenone, a drug with a membrane stabilizing effect, local anesthetic properties, and weak beta-blocking and calcium antagonist effects, has proven to be successful in the treatment of supraventricular and ventricular arrhythmias in adults[1-3]. However, experience with the administration of this drug in children is limited[4,5]. The aim of this study was to assess the antiarrhythmic effects, clinical efficacy and safety of propafenone in the short and long-term management of children with various arrhythmias.

Material and Methods

The prospective study included eighteen children aged between five years and 18 years (median age: 12 years) who had been treated with propafenone for various

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arrhythmias. There were ten females and eight males. The arrhythmias were documented by 12-lead ECG, exercise testing and 24-h Holter monitoring in three children. Electrophysiologic studies were performed in two children (Cases 7 and 13).

Thirteen children had frequent ventricular ectopic activity. Three children had paroxysmal re-entry atrioventricular tachycardia, one with the Wolff-Parkinson-White (WPW) syndrome. One child had paroxysmal atrial tachycardia with frequent supraventricular ectopic activity, and one had paroxysmal atrial flutter or fibrillation. In two children (11.1%) there was associated structural heart disease (Table I). In 13 children (72.2%) up to three (median: 2) conventional anti-

TABLE I: Clinical Details of the Patients

Case	Age (Yrs)	Sex	Arrhythmia	Structural defect	Previous treatment
1	10	F	VES	no	no
2	12	F	VES	no	Epd, Prop, Quin
3	11	F	VES	no	Met, Epd, Quin
4	13	M	VES	no	no
5	15	F	VES	no	Epd, Prop, Dis
6	8	M	VES (intermittent WPW)	no	Epd, Aj, Quin
7	18	F	PRAVT	no	Dig, V, Pind
8	10	M	PRAVT (WPW)	no	no
9	6	M	VES (AES)	no	no
10	13	M	VES	no	Epd
11	13	F	VES	no	Proc
12	11	M	VES	Small VSD	Epd
13	17	F	AFL/FIB	Repaired ASD	Prop, Dig
14	12	M	VES	no	Epd, Quin
15	5	M	VES	no	Epd, Quin, Met
16	14	F	PRAVT	MI-MS	no
17	14	M	VES	no	Epd, Quin
18	6	F	PAT (AES)	no	Dig

VES–ventricular ectopics; WPW–Wolff-Parkinson-White syndrome; PRAVT–paroxysmal re-entry atrioventricular tachycardia; AES–atrial ectopics; PAT–paroxysmal atrial tachycardia; AFL/FIB–paroxysmal atrial flutter or fibrillation; Epd–epdantoin; Prop–propranolol; Quin–quinidine; Met–methoprolol; Dis–disopyramide; Aj–ajmaline; Dig–digoxin; V–verapamil; Pind–pindolol; Proc–procainamide; VSD–ventricular septal defect; ASD–atrial septal defect; MI–mitral insufficiency; MS–mitral stenosis.

arrhythmic drugs had failed to control the arrhythmias. The frequency of paroxysmal tachycardia was assessed prospectively by the use of an incidence score. Improvement in the incidence score by two or more points was considered significant.

Treatment with propafenone was introduced according to the results of previous research. The initial dose of 300 mg/^{2}/day orally or 1 mg/kg intravenously was followed by an oral maintenance dose of 300 mg/m^2/day every eight hours. Treatment was initiated in the hospital or at home, with frequent monitoring. Propafenone was used initially in three patients (16.6%); in one patient (Case 16) during spontaneous tachycardia, and in two patients (Cases 7 and 13) during electrophysiologic studies. Five children had three to five-day periods of continuous ECG monitoring. The dose of propafenone was increased up to 400 mg/m^2/day in order to control the arrhythmias.

Out-patient reassessments were at one, three, and six months and then at appropriate intervals for each patient. These included a review of symptoms, a standard 12-lead ECG, and in some cases, 24-h Holter monitoring.

Results

In-patient treatment

Sinus rhythm was achieved in all three children who had been given propafenone during their episodes of tachycardia. There was no clinically apparent negative inotropic effect in these children. All the children treated for tachycardias were free of arrhythmias on the day of discharge. Antiarrhythmic agents were continued in the four children already on treatment at the time of inclusion in the study. Propafenone was discontinued in one patient.

Ventricular ectopics were recorded daily in thirteen children and in seven patients their frequency decreased by more than 75 percent.

The median daily dose of propafenone at discharge was 300 mg/m^2/day (range: 250-400 mg/m^2/day; Table II).

Ventricular ectopics (thirteen children)

Seven patients (Cases 4, 6, 11, 12, 14, 15, 17) had total suppression of ventricular ectopic activity at their last review. These patients were not on any additional antiarrhythmic agents (Fig. 1). The other six children with ventricular ectopics continued to experience frequent ectopic activity, and they were withdrawn from propafenone treatrnent.

TABLE II: Follow-up of the Patients

VES case	Dose ($mg/m^2/day$)	Other Drugs	Outcome	Follow-up (mo.)
1	300	no	Failure	10 (W/D)
2	350	no	Failure	12 (W/D)
3	300	no	Failure	8 (W/D)
4	250	no	Success	12
5	400	no	Failure	4 (W/D)
6	275	no	Success	16
9	250	no	Failure	2 (W/D)
10	300	no	Failure	3 (W/D)
11	250	no	Success	4
12	300	no	Success	6
14	350	no	Success	7
15	250	no	Success	8
17	300	no	Success	10
PRAVT case	**Dose ($mg/m^2/day$)**	**Other Drugs**	**Outcome**	**Follow-up mo.**
7	350	no	Success	7
8	300	no	Success	6
16	250	no	Success	4
PAT or AFL/FIB case	**Dose ($mg/m^2/day$)**	**Other Drugs**	**Outcome**	**Follow-up mo.**
13	400	Dig	Failure	4 (W/D)
18	300	no	Partial	3

VES–ventricular ectopics; PRAVT–paroxysmal re-entry atrioventricular tachycardia; PAT–paroxysmal atrial tachycardia; AFL/FIB–paroxysmal atrial flutter or fibrillation; Dig–digoxin; W/D–withdrawn.

Paroxysmal re-entry atrioventricular tachycardia (three children)

Treatment with propafenone was successful in all three children. They were not on any additional antiarrhythmic agents. 24-h Holter-monitoring was planned to reassess the suppression of the arrhythmia. One case with the WPW syndrome (Case 8) continues to have anterograde conduction via the accessory pathway. Intravenous propafenone was successful in one patient (Case 16) during the episodes of tachycardia (Fig. 2).

Paroxysmal atrial tachycardia, flutter or fibrillation (two children)

One child (Case 13) responded poorly to treatment, and despite the addition of digoxin to the regimen due to troublesome episodes of tachycardia, was withdrawn from the study after four months. Another child (Case 18) in which partial suppression of arrhythmia was observed at follow-up also demonstrated,

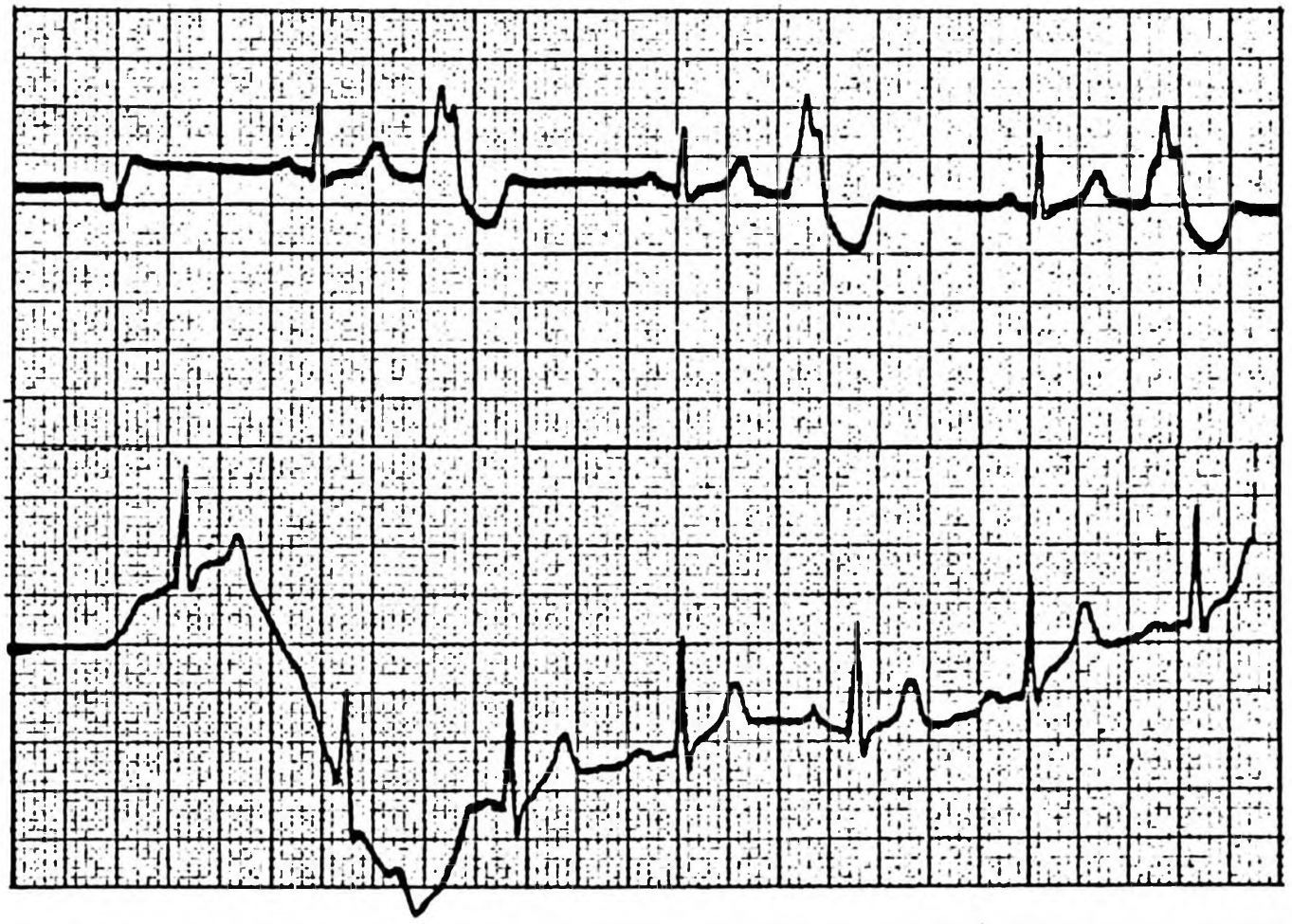

Fig. 1. The recording demonstrates a ventricular bigeminy (Lower III b). After treatment with propafenone, ventricular ectopic activity disappeared.

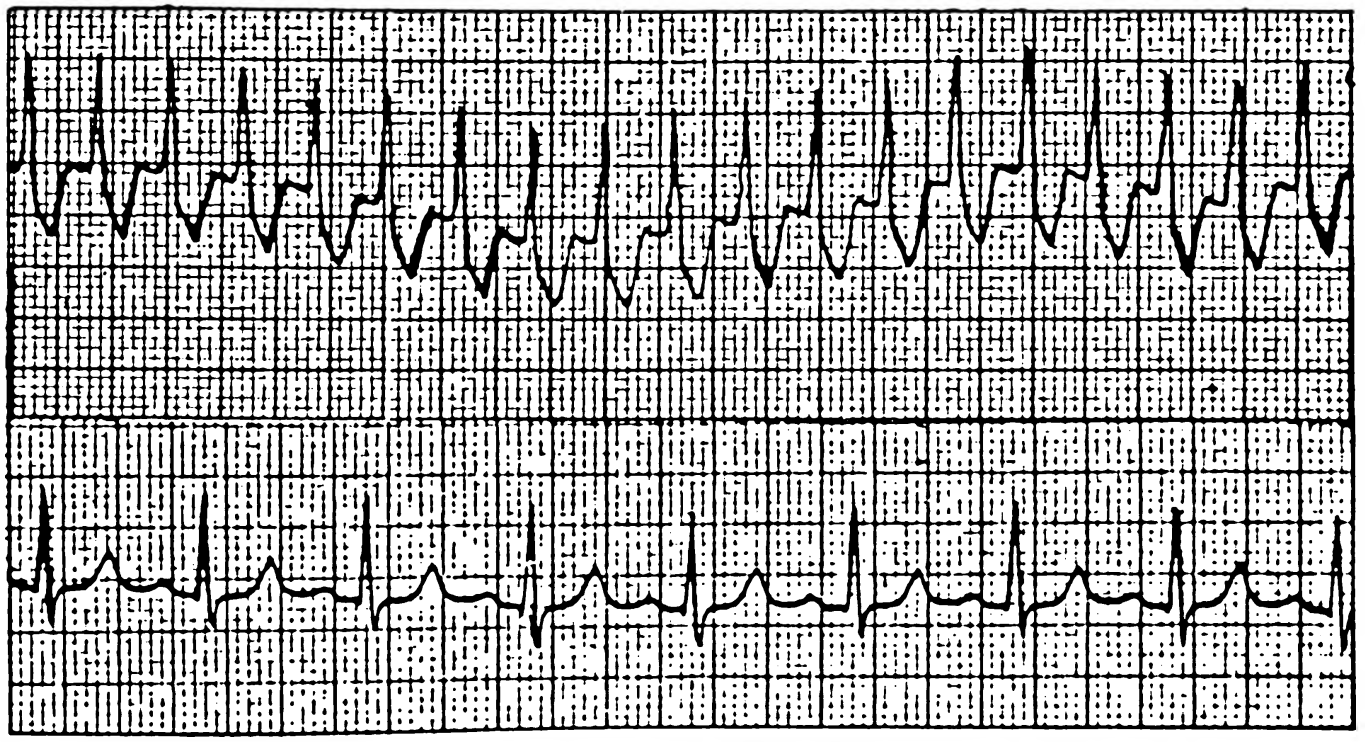

Fig. 2. Paroxysmal re-entry atrioventricular tachycardia (Case 16) terminated in a few minutes time by the administration of intravenous propafenone.

to some degree, disappearance of premature atrial contractions during 24-h Holter monitoring and recently total suppression of the tachycardia.

Side Effects and withdrawals

Treatment with propafenone did not cause major arrhythmogenesis or side effects. Seven children were withdrawn from the study because they had failed to respond to treatment (Table II).

Discussion

Propafenone is a new antiarrhythmic agent with Vaughan Williams Class Ic action[1-3]. This drug has various effects on the cardiac system and ventricular myocardium[6]. The treatment of arrhythmias in children raises particular problems related to pharmacokinetics, the nature of arrhythmias, growth, drug administration and compliance. There is always a need for new, effective and safe antiarrhythmic agents which can be used in children. Experience has shown propafenone to be an effective agent in the management of adult arrhythmias since it is effective in the control of ventricular arrhythmias and those associated with preexcitation syndromes[7-10].

In this study, we report the use of propafenone in children, in whom it was most effective against supraventricular arrhythmias when used alone. Propafenone therapy was successful in seven of thirteen (53.8%) children with ventricular ectopic activity who had a long-term follow-up. In the other six cases, no significant reduction of ventricular ectopic activity was observed. The use of propafenone has been evaluated in adult patients with frequent ventricular ectopics who responded to therapy[6,7,9]. Various studies report that suppression of ventricular ectopic activity by more than 85 percent was achieved in 60 to 80 percent of cases[6,7]. Our six patients who were withdrawn from propafenone treatment had no symptoms, and dose increments had not been considered. In six of the seven children in whom suppression of ventricular ectopic activity was achieved with propafenone, conventional antiarrhythmic drugs had failed to control the arrhythmias. This result shows the efficacy of propafenone on clinically significant ventricular ectopic activity.

The administration of propafenone alone controlled the paroxysmal re-entry atrioventricular tachycardia in three of the children in our study. Two children were difficult to manage because they had been previously resistant to conventional arrhythmic agents. Musto et al[5] reported the electrophysiologic effects and clinical efficacy of propafenone in children with supraventricular tachycardia and found that propafenone prolonged retrograde conduction in all their patients and blocked or prolonged anterograde conduction in 11 of 18 patients with accessory connections. Musto et al[6] also reported the long-term results of propafenone

therapy which was successful in three of four children with paroxysmal re-entry atrioventricular tachycardia[5]. In this study, the dosage of propafenone given in two or three divided doses was 14.3±3.8 mg/kg/day. In the study reported by Dressler et al[4], propafenone in a dose of 300 mg/m^2/day was administered three to four times a day and was effective in 85.7 percent of 35 patients[4]. This recent finding, together with ours, emphasizes the efficacy of propafenone in treating children with paroxysmal re-entry atrioventricular tachycardia.

In one child with paroxysmal atrial flutter or fibrillation that we studied, treatment failed despite the combination with digoxin. Coumel et al[9] found that as an anti-arrhythmic agent, propafenone was less effective than quinidine or amiodarone, but Connolly and Hoffert[11] reported the successful use of propafenone in the treatment of atrial flutter or fibrillation in their long-term studies. In another child in our study with paroxysmal atrial tachycardia, the episodes disappeared with the administration of this drug but complete suppression of atrial ectopic activity could not be achieved.

Intravenous propafenone was effective in controlling supraventricular tachycardia in three children (Cases 7, 13 and 16). Recent studies have shown the efficacy of propafenone in controlling supraventricular tachycardias which were no longer inducible or nonsustained during electrophysiological study[5,10].

The lack of observed side effects in our study indicates that propafenone is a promising anti-arrhythmic agent which has been evaluated in recent reports. Despite this finding, some studies have revealed a negative inotropic effect of propafenone, a fact that should be considered when this drug is used in patients with depressed left ventricular function[1-3].

In conclusion, the administration of propafenone is effective and safe in children and may be used in the management of supraventricular and ventricular arrhythmias for which a Class 1 anti-arrhythmic drug is required.

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DETERMINATION OF ARM FAT AREA AND ARM MUSCLE AREA NORMS IN CHILDREN 6-12 YEARS OF AGE IN BURSA*

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Summary: Günay Ü, Sapan N, Salih C, Doğruyol H. (Departments of Pediatrics and Pediatric Surgery, Uludağ University Faculty of Medicine, Bursa, Turkey). Determination of arm fat area and arm muscle area norms in children 6-12 years of age in Bursa. Turk J Pediatr 32: 93-100, 1990.

Since arm fat area and arm muscle area measurements are said to assess the calorie and protein reserves in the body more accurately than triceps skinfold thickness measurements, we decided to use this system on 1497 girls and 1651 boys who were pupils in elementary schools in Bursa. From the data obtained, percentile norms for the children aged between 6-12 were calculated and percentile curves were drawn. The data that we collected can be used in future nutritional surveys. *Key words: malnutrition, arm fat area, arm muscle area.*

Anthropometric measurements are used universally for the evalution of growth in childhood. Although genetic factors influence somatic growth, environmental factors, such as nutrition, effect somatic growth too. Inadequate calorie and protein intake causes varying degrees of growth and developmental delays. Percentile curves are very useful in the evaluation of growth in children. In field investigations, the simplest means to assess nutritional status is body-weight for age measurement. The growth of a child should not only be considered an increase in body weight, but conditions of various body components such as fat and muscle masses must also be taken into account. For that reason measurements of midupper arm circumference (MUAC) and triceps skinfold thickness (TST) are very important since they reflect both fat and muscle masses. Subcutaneous fat layers and muscle masses are considered to be indirect indicators of calorie and protein reserves, respectively[1].

For these reasons, MUAC and TST are used in assessing the nutritional status of children[1-7]. But recently arm fat area (AFA) and arm muscle area (AMA)

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measurements have been found to determine calorie and protein reserves better than MUAC and TST. Few studies on this subject have been reported in the literature[4-8], and to our knowledge no reports have been published in Turkey.

Material and Methods

Over a period from March 1, 1988 to April 30, 1988, elementary school pupils from the city of Bursa were studied. Of the 1952 classes from 87 elementary schools, 75 classes were chosen at random (3168 children). There were 1497 girls and 1671 boys.

MUAC and TST were measured in all of the children. From this data AFA and AMA were calculated using the following equations[4-8].

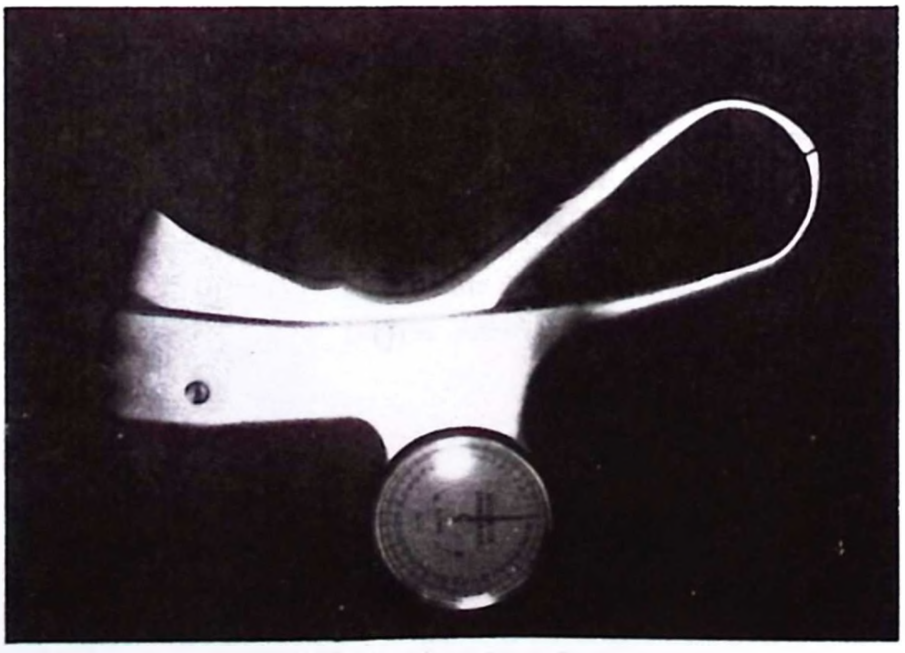
$$\text{Arm area (AA)} = \text{MUAC}^2 / 4\ \pi$$
$$\text{AMA} = (\text{MUAC} - \pi\ .\ \text{TST})^2/4\ \pi$$
$$\text{AFA} = \text{AA} - \text{AMA}$$

The ages of the children were obtained from their birth certificates and whole ages were taken into consideration.

The same investigator took measurements from the midpoint between the tip of the acromion and olecranon processes in the flexed left arm of all the patients.

MUAC and TST measurements were taken from the same point of the left arm which was extended and hanging freely.

MUAC was measured with a plastic tape, the accuracy of which was controlled daily with a steel tape. TST was measured with a Holtain Skinfold Caliper designed to give a constant pressure of 10 g/sq mm (Holtain LTD., Crosswell, Crymych, Dyfed SA 41 3 UF, U.K.) (Fig. 1). Readings were done at the 15th second



Holtain Skinfold Caliper

after the application of the caliper[7]. The millimeter was used as the unit of measurement. Percentiles for all age groups were calculated[9]. In order to show the results in graphical form, the relationship between each pair of variables was examined[9]. Since there was a linear relationship between age, AFA and AMA, smooth curves were drawn using a linear regression formula[9].

Results

Table I illustrates the distribution of boys and girls in all age groups. The proportion of girls and boys in each age group was very similar.

TABLE I: Distribution of Girls and Boys in All Age Groups

Age (yrs)	Girls n (%)		Boys n (%)		Total
6	179	(45.2%)	217	(54.8%)	396
7	265	(47.0%)	298	(53.0%)	563
8	294	(46.8%)	333	(53.2%)	627
9	320	(51.3%)	303	(48.7%)	623
10	290	(46.6%)	332	(53.4%)	622
11	120	(44.1%)	152	(55.9%)	272
12	20	(44.6%)	36	(55.4%)	65
Total	1497	(47.2%)	1671	(52.8%)	3168

A. AFA measurements :

AFA percentile norms of girls and boys are shown in Tables II and III; the curves are shown in Figs. 1 and 2, respectively. There was a linear relationship found between age and AFA in the 6-12 year-old age groups.

TABLE II: Linear Percentile Norms of AFA (mm^2) of Girls

Age (yrs)	Percentile (%) 5	10	25	50	75	90	95
6	340.6	401.7	484.6	613.0	758.9	916.2	1030.3
7	381.0	438.4	524.1	656.4	818.7	1007.3	1149.6
8	421.3	475.0	563.5	699.9	878.5	1098.4	1269.0
9	461.6	511.7	603.0	743.3	938.4	1189.4	1388.3
10	502.0	548.3	642.5	786.7	998.2	1280.5	1507.6
11	542.3	584.9	682.0	830.2	1058.0	1371.5	1627.0
12	582.6	621.6	721.5	873.6	1117.9	1462.6	1746.3

TABLE III: Linear Percentile Norms of AFA (mm^2) of Boys

Age (yrs)	Percentile (%) 5	10	25	50	75	90	95
6	317.2	381.2	460.8	563.3	647.6	769.7	832.1
7	337.5	396.4	478.2	589.0	701.2	858.1	953.4
8	357.8	411.6	495.7	614.7	754.8	946.5	1074.8
9	378.1	426.9	513.1	640.4	808.3	1035.0	1196.1
10	398.4	442.1	530.5	666.1	861.9	1123.4	1317.5
11	418.7	457.3	548.0	697.8	915.4	1211.9	1438.8
12	439.0	472.5	565.4	717.5	969.0	1300.3	1560.2

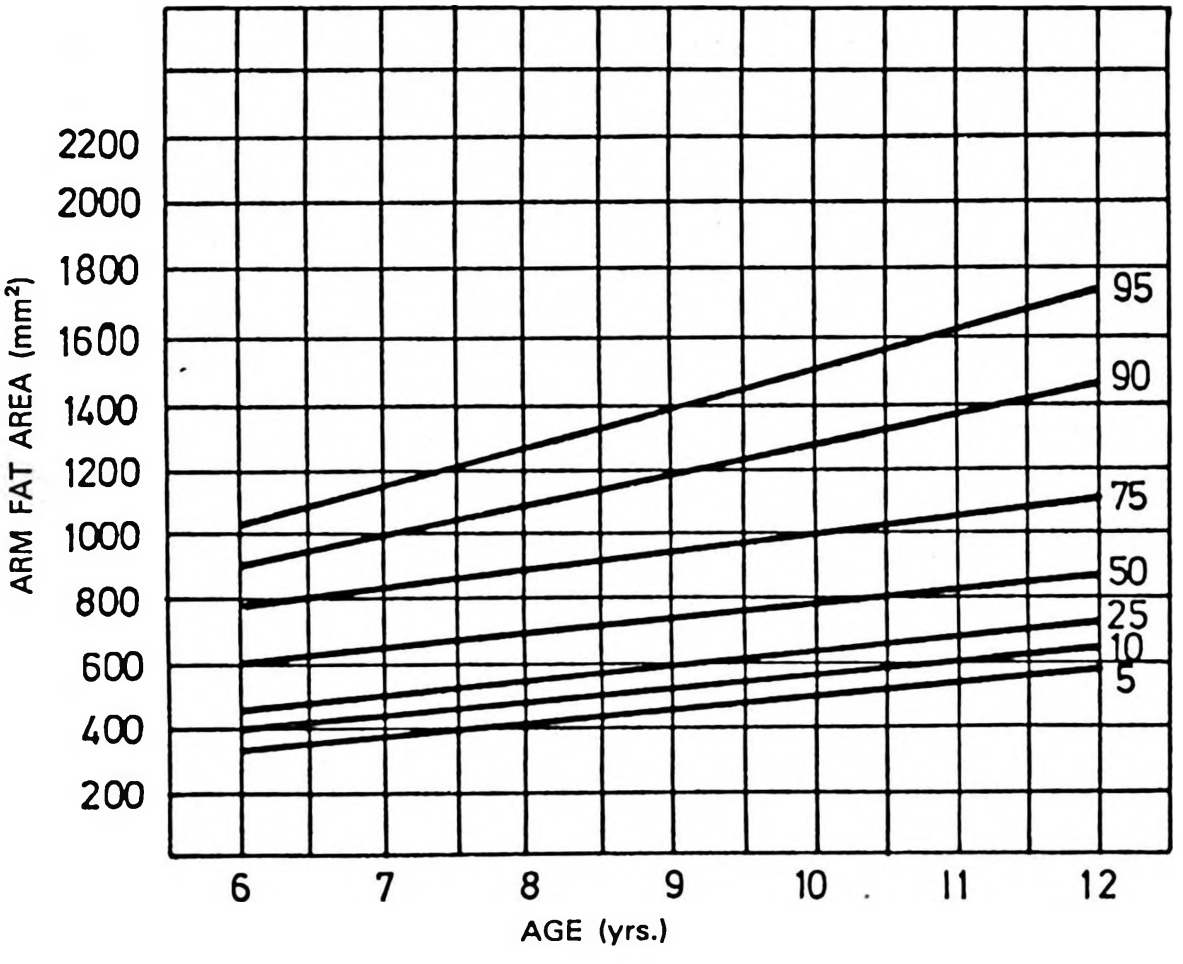


Fig. 1: Smooth percentile curves of AFA of girls (aged 6-12 years).

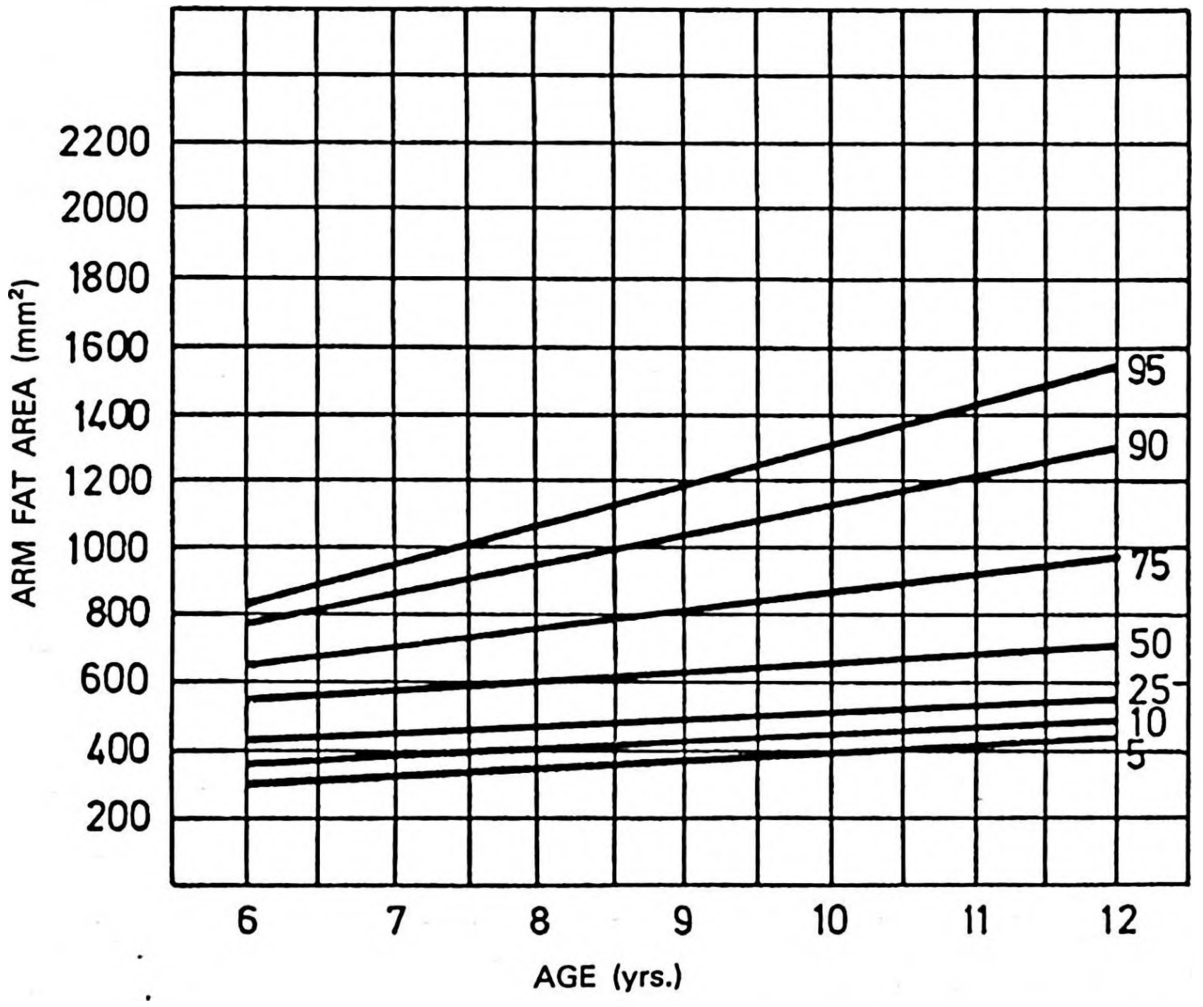


Fig. 2: Smooth percentile curves of AFA of boys (aged 6-12 years).

B. AMA measurements :

AMA percentile norms of girls and boys are shown in Tables IV and V; the curves are shown in Figs. 3 and 4, respectively. A linear relationship was also found between AMA and age.

TABLE IV: Linear Percentile Norms of AMA (mm^2) of Girls

Age (yrs)	Percentile (%) 5	10	25	50	75	90	95
6	1242.1	1348.9	1483.6	1654.2	1772.0	1962.7	2077.7
7	1324.7	1431.1	1577.1	1761.1	1927.5	2132.8	2272.4
8	1407.4	1513.3	1670.5	1868.1	2082.9	2302.8	2467.1
9	1490.0	1595.5	1763.9	1975.1	2238.4	2472.9	2661.8
10	1572.6	1677.7	1857.4	2082.0	2393.9	2942.9	2856.5
11	1655.3	1759.9	1950.8	2189.0	2549.4	2812.9	3051.2
12	1737.9	1842.1	2044.2	2295.9	2704.8	2983.0	3245.9

TABLE V: Linear Percentile Norms of AMA (mm^2) of Boys

Age (yrs)	Percentile (%) 5	10	25	50	75	90	95
6	1305.9	1381.1	1532.0	1696.8	1893.6	2083.3	2161.3
7	1417.9	1493.4	1642.9	1816.6	2020.7	2229.4	2342.4
8	1529.9	1605.7	1753.8	1936.5	2147.8	2375.5	2523.6
9	1641.9	1718.0	1864.7	2056.3	2274.9	2521.6	2704.7
10	1753.9	1830.3	1975.7	2176.1	2402.0	2667.7	2885.8
11	1865.9	1942.6	2086.6	2296.0	2529.1	2813.8	3067.0
12	1977.9	2054.9	2197.5	2415.8	2656.2	2959.9	3248.1

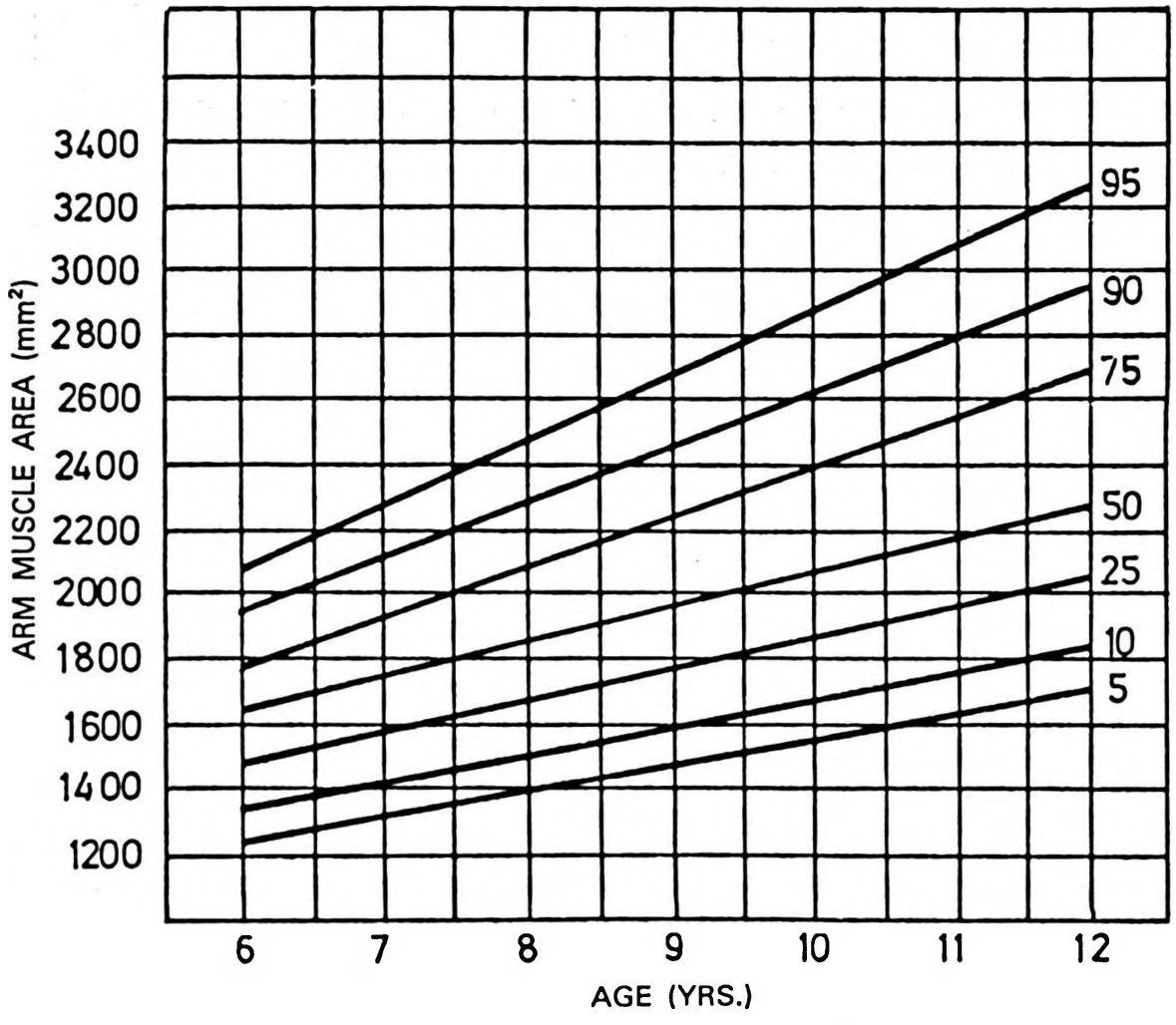


Fig. 3: Smooth percentile curves of AMA of girls (aged 6-12 years).

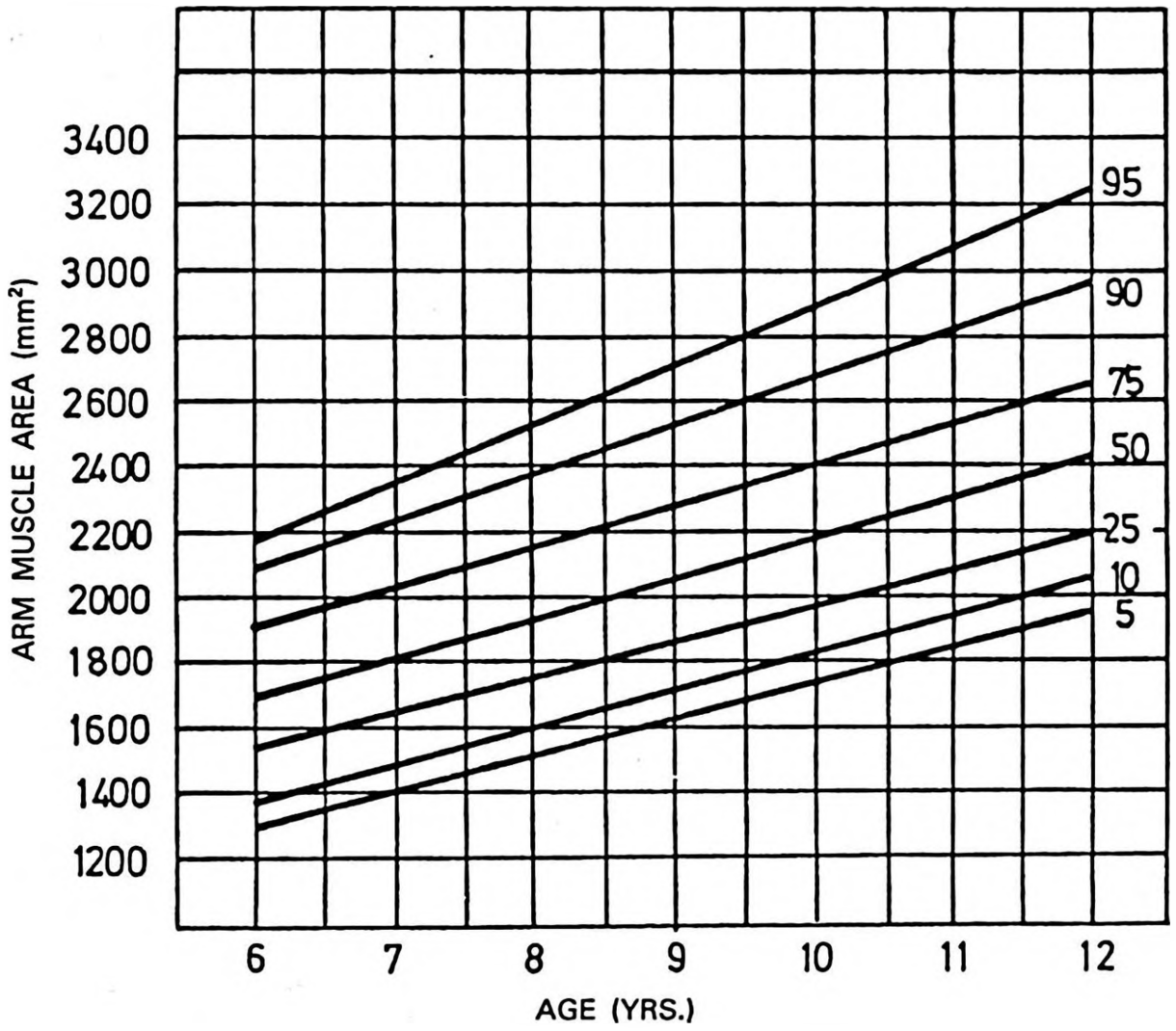


Fig. 4: Smooth percentile curves of AMA of boys (aged 6-12 years).

Discussion

The most widely used anthropometric measurement in the evaluation of growth is weight for age. But the World Health Organization has recommended that not only weight for age, but also weight for height should be used[10]. However, none of these measurements gives information about the protein and fat reserves of the body[11]. Especially in severe malnutrition, because of water retention in the extracellular compartment, the total amount of body water increases abnormally, resulting in a false increase in body weight. But at the same time calorie and protein reserves may be decreased[8]. In these kinds of situations, body weight measurements give erroneous results.

The main protein reserve in the body is the skeletal muscle mass, and the calorie reserve is the subcutaneous fat layer. Because of the reasons stated above, MUAC and TST measurements offer more accurate information, since they reflect both the muscle mass and the subcutaneous fat layer[1].

Many authors believe that in assessing the nutritional status of children, MUAC and TST measurements should be used[6,12]. But in recent years, results of some studies have shown that AFA and AMA measurements reflect the status of the muscle mass and subcutaneous fat layers more accurately than MUAC and TST measurements[4-7].

In two studies carried out by Frisancho et al[4,6] in the United States, one involving 12,496 subjects aged 0-44 years, and the other involving 19097 subjects aged one to 74 years, they observed that the AFA and AMA measurements were more accurate indicators of body protein and calorie reserves than the MUAC and TST measurements. Sann et al[7] performed a study on 100 infants in France and reached the same conclusion.

We presented the AFA and AMA percentiles of girls and boys aged between 6-12 years who reside in Bursa. This data can be used in future nutritional surveys in Turkey.

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INJURIES OF THE VULVA AND VAGINA IN CHILDHOOD*

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Summary: Okur H, Zorludemir Ü, Yücesan S, Olcay I. (Department of Pediatric Surgery, Çukurova University Faculty of Medicine, Adana, Turkey). Injuries of the vulva and vagina in childhood. Turk J Pediatr 32: 101-106, 1990.

Injuries of the vulva and vagina are relativly rare in children. Over a seven-year period, we treated 45 girls. The most common etiologic factor in our study group was trauma. While 28 had only vulvar lesions, the rest had injuries of both the vulva and vagina. Thirty-two children were treated surgically for only vulvar and vaginal injuries. However, additional organ treatment was mandatory for 13 children. *Key words: vulvar injury, vaginal injury.*

There are very few documented cases in the literature describing injuries of the vulva and vagina (V V)[1-10]. Over a seven-year period, we treated 45 children with V V injuries at the Çukurova University Medical Center. This figure amounts to 7.2 % of the children who were admitted with the diagnosis of trauma in the same period. These findings prompted us to study this rare and interesting type of trauma.

Material and Methods

The study population comprised forty-five patients with V V injuries who had been admitted and treated in the Department of Pediatric Surgery between the years 1978 and 1985. Children experiencing minor injuries i.e., crushing and hematomas in which surgical treatment was not required, were not included in this study.

All clinical reports of the 45 children were reviewed within the framework of the following parameters: age, etiology, time of admission, symptoms, physical and laboratory findings, traumatic lesions, surgical approaches, complications and mortality, the relation of organ and/or system injury to complications and mortality.

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Results

Etiology : Only 38 (84.4 %) of the 45 children presented with information about the etiology of their injury in their clinical files. Twenty of them had fallen from a height and 15 had been injured in a traffic accident. One patient was injured when a wall collapsed, and a six year-old girl was raped. The injury in the newborn baby was due to birth trauma. All the children who had fallen from a height had no other history which would have caused V V injury. Forty percent of the children had fallen from roofs.

Age : Our youngest patient was a four-day-old newborn, eight patients (17.7 %) were between 1-4 years of age, 32 patients (71.3 %) were between 5-9 years of age, and four patients (8.8 %) were between 10-13 years of age.

Admission time : The fastest admission to the hospital was ten minutes and the slowest was six days after the injury. Fourteen (31.1 %) of the patients arrived within the first hour, twelve (26.2 %) within 1-6 hours, eight (18.0 %) within 6-24 hours and four (8.8 %) within 1-6 days after the injury. The admission time of the remaining seven patients was not recorded.

Symptoms and physical findings : In most of the patients pain was determined in the genital region, while in the rest it was located in the pelvic region, the extremities, and in the abdomen. Upon examination in the emergency room, hematuria was noted in five patients, vaginal bleeding in 16, respiratory difficulty in one, hypotension (60-40 mm Hg and below) in seven and tachycardia in15.

Laboratory findings : Hemoglobin (Hb) levels were above 10 g/dl in 36 patients and below that level in nine patients. Three urethra ruptures, one bladder neck rupture, and one perivesical hematoma were diagnosed by IVP and cystogram studies. X-ray studies demonstrated the presence of lower extremity and/or pelvic fractures in seven patients.

Traumatic lesions : While 32 (71.1 %) of the children had only V V injuries, the rest (28.9 %) also had additional organ injuries.

Vulvar injuries : Twenty-eight of the 45 children had only injuries of the vulva. In these patients fifty-two injuries were demonstrated in various areas of the vulva (Table I). Eighteen (34.9 %) of the lesions occurred in the labium minus. This was followed by injuries of the hymen (23 %), labium majus (15.3 %), clitoris (15.3 %) and comissura posterior (11.5 %).

Extensive vaginal lacerations : The posterior fornix was the most injured part of the vagina and laceration was found in eight children. Other vaginal injuries were as follows : vaginal rupture in two children, anterior wall laceration in one child and right fornix laceration in one child. In addition to the vagina, an injury to the uterus was also found in one child.

TABLE I: Localization of Vulvar Injuries in 28 Patients

Localization	Number of Patients (%)
Labium minus	18 (34.9%)
Hymen	12 (23.0%)
Labium majus	8 (15.3%)
Clitoris	8 (15.3%)
Posterior comissura	6 (11.5%)

Additional organ injuries : In our study group several additional organ injuries were also observed in 13 children (28.9 %). Eight patients had anointestinal, seven skeletal, and five urologic injuries while three children had extensive skin lacerations. Left diaphragm laceration, lung laceration, right femoral artery and vein injuries were separate operative findings.

Surgical approaches : While 32 of the 45 patients had only V V repair, 13 patients also had additional surgical procedures as follows : colostomy (6),cystostomy and urethral anastomosis (4), anoplasty (3), cytostomy (2), diaphragmatic repair (1) and suture of the sigmoid colon serosa and mesentery (1) (Table II).

TABLE II: Surgical Approaches in 45 Patients With Injuries of the Vulva and Vagina.

Surgical Approach		Number of Patients (%)
Vulva and Vagina Procedures		32 (71%)
Simple Suturing	8	
Extensive Surgery	24	
Additional Organ Procedures		13 (29%)
Colostomy	6	
Cystomy and Urethral anastomosis	4	
Anoplasty	3	
Cystostomy	2	
Diaphragmatic repair	1	
Suture of the sigmoid Serosa and mesentery	1	
Total		45 (100%)

Complications and mortality : Four complications (8.8 %) were encountered: in the early postoperative period, wound infection occurred in two children; one child had a leg amputated, and urinary incontinence developed in one child in the late postoperative period. There were no other late complications or functional or cosmetic problems in the one-eight years that followed.

There were only two mortalities in the group. One of the deaths was related directly to the trauma. The child had V V destruction and extensive tissue loss. The other patient died in the early postoperative period of colostomy closure, due to respiratory arrest.

The correlation between time of admission, complications and mortality : There was no clear correlation between admission time and complications. Three of four children with complications were admitted to the hospital shortly after treatment, which was an elective procedure.

The correlation between additional organ injury, complications and mortality : Additional organ injuries were encountered in three of the four children who developed complications. One of the deaths in this group had multiple system injuries due to a traffic accident.

Discussion

Injuries of the vulva and vagina are not common in children, and thus, few of these cases have been reported in the literature. However, Wynne[10] has reported 33 such childhood cases in his series, and Rush and Milton[8] have described a total 36 children and adults with V V injuries.

In this study nearly half of the patients (40 %) who had fallen from a height fell from roofs. The roofs of houses in the small towns and villages of the Çukurova region are smooth and built of concrete. They are used for various purposes, such as sleeping areas on hot summer nights. All the injuries that resulted from falling from heights happened during the warm seasons of the year, which means that a certain type of serious trauma occurs in the southern part of the country. On the other hand, the etiology of V V injury in the newborn baby in our study group is a very rare type of trauma. It was due to the vaginal examination performed on the mother by the obstetrician during the baby's breach presentation. This situation can be considered a prenatal V V injury which has never been reported before.

In all series of V V injuries, rape takes first place in the etiology. Wynne[10] reported that 24 (72.7 %) of his patients were injured as a result of rape. Rush and Milton[8] described 25 patients with coital injuries, 14 of which were the result of rape. It is confirmed that 20,000-50,000 girls are raped in the U.S.A every year, and that 67,131 cases of rape were reported in 1978[9]. In that report, the ages of the victims varied between three months and 90 years. In Wynne's series[10], the

youngest patient was 15 months, and the oldest was 12 years old. In our study group there was only one child who had been raped. She was a six-year-old girl who was brought to the hospital 32 hours after the act had occurred. She had a perineal laceration extending from the posterior wall to the anus. None of our other patients gave a history of rape either on admission or during treatment.

The mean age of our patients was 6.24 years. Thirty-two (71 %) of the children were five to nine years of age. Thus, most of the subjects in this study were children engaged in play activities. These findings explain why etiologic factors in most of the cases were falls and traffic accidents. Therefore, to avoid this serious problem, school transportation vehicles should be carefully examined, a proper program in traffic education instituted, sufficient playgrounds constructed, and ralling should be installed around roofs.

In our study, most of the injuries were caused either by a fall from a height or by a traffic accident. Some of the injuries resulted in lower extremity and/or pelvic fractures. These findings once again demonstrate the close relation between fractures and V V injuries. The ten-year-old patient described by Ikpeme and Morrison[11] is a typical example of a V V injury resulting from a pelvic fracture. In one series reported, two of 16 patients with open pelvic fractures, and four of 114 patients in another group with pelvic fractures also had V V injuries[12].

Clinical and laboratory findings play an important role in the treatment of V V injuries. An early and careful examination must be carried out on these children who have genital organ injuries. It is our policy to perform the clinical examination under general anesthesia, which not only provides a detailed and easy examination but also prevents psychologic damage which might lead to sexual problems when the child reaches adulthood. Of the 28 patients in our study with vulvar injuries, 14 of the children had first degree, 11 had second, and three had third degree lacerations. These findings are almost the same as the findings reported by Wynne[10]. On the other hand, we also had two children with abdominal penetration of vaginal rupture, and two with complete destruction of the vulva and vagina. The last type of injury is the most serious, and the two patients with this injury are the first ever to be reported in the literature.

Rush and Milton[8] and Wynne[10] believe that delays in admission play a considerable role in development of complications. About 34% of the patients in Wynne's series[10] were hospitalized between one and seven days following the injury. We found no correlation between delays and complications in our patients. In our study group, three of the four children who developed complications were admitted shortly after the injury occurred.

Additional organ injuries cannot be ignored in our series. Thirteen of the children had additional organ and/or system injuries which resulted in complications in

three and in death in one. These findings, once again, underline the importance of traffic accidents and falls from heights in the etiology.

Different mortality rates have been reported in V V injuries. Rush and Milton[8] and Wynne[10] reported no mortality in their series. We reported only one death, which constitutes a mortality rate of 2.2 percent. This patient was brought to the hospital nine hours after a traffic accident in hypovolemic shock. Since most of our children were admitted with additional organ and/or system trauma, it is our opinion that complications and mortalities in this study were not directly caused by V V injuries.

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FATAL INFECTIOUS MONONUCLEOSIS IN A FAMILY*

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Summary: Sanal Ö, Kale G, Ersoy F, Göğüş S, Coşkun Y, Çağlar M, Berkel A. İ. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Fatal infectious mononucleosis in a family. Turk J Pediatr 32: 107-115, 1990.

Two male siblings, one aged five and a half months (SB), and the other aged six months (VB), with fatal infectious mononucleosis phenotype of the X-linked lymphoproliferative syndrome, which resulted in the death of both infants, are presented. Both patients had been healthy, one until the age of five and a half months, and the other until the age of six months. Then, they developed a maculopapular rash, hepatosplenomegaly and lymphadenopathy. In one sibling, the serum IgG level was low, the IgM and IgA levels were high, and the proportion of E-rosette forming cells (E-RFC) and in vitro proliferative response to PHA were normal. In the other sibling, however, the serum IgG level was normal, the IgM and IgA levels were high and the stimulation index for proliferative response to PHA was reduced due to increased spontaneous blastogenesis. Anti-EBV antibodies were negative in both siblings, except for the IgM anti-VCA in V.B. A lymph node specimen could be studied in one infant and was found to be positive for the EBV genome. Postmortem histopathological findings included the absence of cortico-medullary differentiation and identifiable Hassal's corpuscles in the thymus and depletion of T-dependent regions of lymph nodes and spleen in V.B. Atypical mononuclear cell infiltration was detected in the portal areas of the postmortem liver biopsy in S.B. *Key words: fatal infectious mononucleosis, X-linked lymphoproliferative syndrome, thymic pathology.*

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X-linked lymphoproliferative syndrome (XLP), first reported by Purtilo et al[1], may occur as various phenotypes, i.e. chronic and fatal infectious mononucleosis (IM), acquired agammaglobulinemia, aplastic anemia, and B cell non-Hodgkin's lymphoma. Since its original description, many cases of this disease have been diagnosed in various countries[2,3]. We report two male siblings with fatal infectious mononucleosis phenotype who died, one at the age of six months, and the other at the age of six and a half months.

Case Reports

Case 1

S.B. was first seen at the age of five and a half months at the Hacettepe University Children's Hospital with complaints of high fever, generalized rash, diarrhea and vomiting.

He was healthy until about ten days prior to admission, when he developed a high fever, and then a skin rash. He was a well developed boy (weight 9.0 kg: height 71 cm). Physical examination revealed a febrile child with a maculopapular rash covering the entire body including the face and extremities, oral moniliasis, whitish spots on the tonsils, hepatosplenomegaly, and cervical lymphadenopathy (Table I). The fever remained high during hospitalization, and he developed melena. He died on the third day after his admission, about twenty days after onset of the illness.

TABLE I: Clinical Findings

SB	VB
AGE 5 1/2 MONTHS	AGE 6 MONTHS
W 9.0 kg, H 71 cm	W 9.0 kg, H 68 cm
FEVER	FEVER
ORAL MONILIASIS	MEMBRANE ON TONSILS
TONSILLITIS	AXILLARY LYMPHADENOPATHY
CERVICAL LYMPHADENOPATHY	HEPATOSPLENOMEGALY
HEPATOSPLENOMEGALY	MENINGEAL SIGNS AND
DIED ON THE 3rd HOSPITAL	JAUNDICE DEVELOPED
DAY, 20 DAYS AFTER ONSET	DIED ON THE 20TH HOSPITAL
OF ILLNESS	DAY, 30 DAYS AFTER ONSET
	OF ILLNESS

Case 2

V.B., the brother of S.B., was admitted to the hospital at the age of six months with the chief complaints of fever and skin rash. He was well developed and healthy until seven days prior to admission when he developed a skin rash which started from the shoulders and spread to the chest. Physical examination revealed

a child with a high fever, a maculopapular rash on the chest, abdomen, shoulders and upper arm, a membrane on the tonsils, axillary lymphadenopathy, and hepatosplenomegaly (Table I). His height was 68 cm and weight 9 kg. Since bacterial infection could not be ruled out, antibiotics, were started, and because of the low serum IgG level (<200 mg/dl), he was given gammaglobulin (1.8 mg/kg im). Despite this treatment, his condition gradually deteriorated, and he began to have seizures. Examination of the cerebrospinal fluid revealed mononucleated cells (55/mm^3), protein concentration of 85 mg/dl, and glucose 52 mg/dl. His hemoglobin level fell to 6 g/dl, and irradiated whole blood (10 cc/kg) was transfused. He developed jaundice and his condition deteriorated further. He died on the 20th day of hospitalization.

The parents of the siblings were unrelated and healthy. Two sisters, one seven years old and the other four years old were healthy. The second-born child in the family, a five-month old boy, had also died of an infectious disease with high fever (Fig. 1). Two aunts and four uncles on the paternal side are alive and healthy but a paternal uncle had died of pneumonia at the age of six months. Five aunts and six uncles on the maternal side had died of undetermined causes at ages ranging from 18 days to three and a half years.

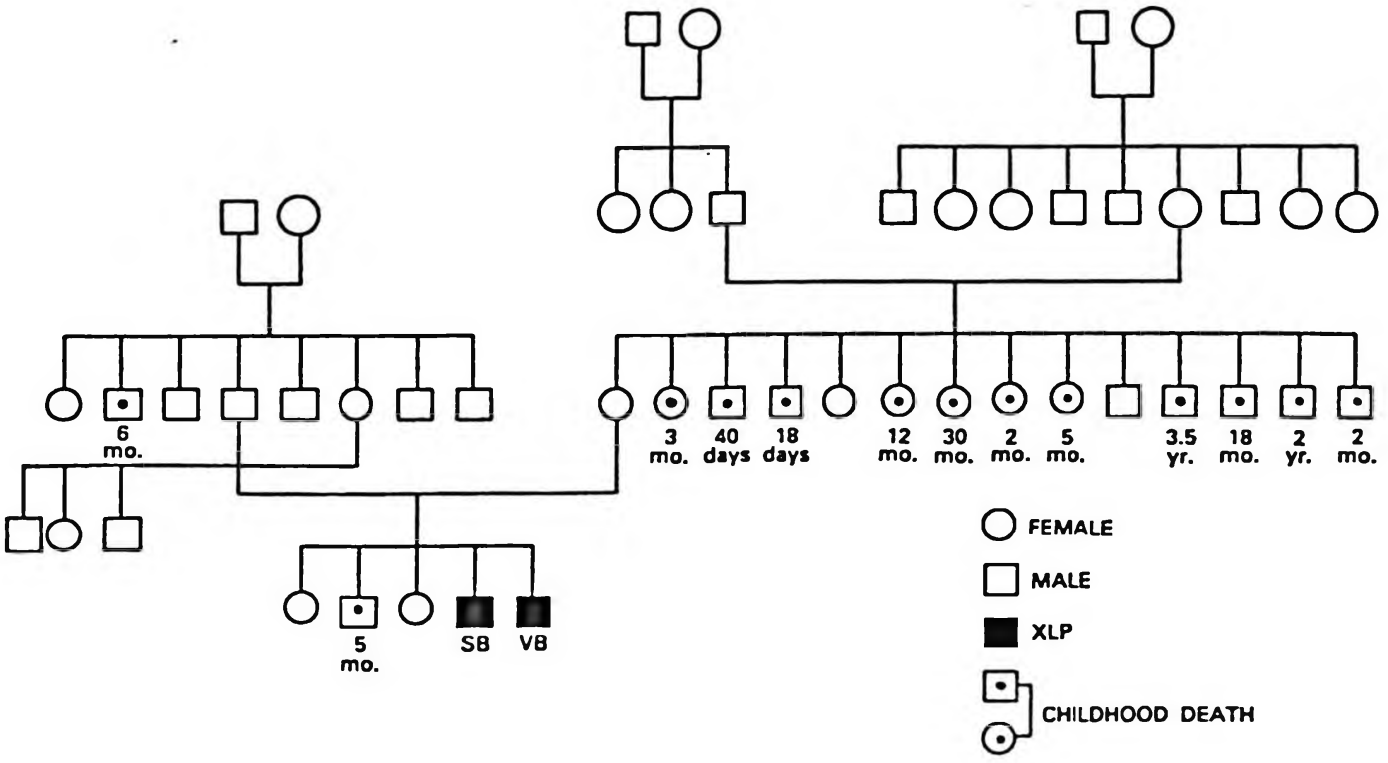

Fig. 1: Family Pedigree

Laboratory Findings :

Peripheral blood smears revealed atypical lymphocytosis and both patients developed neutropenia and thrombocytopenia (Table II). Case 1 had blast-like cells on the bone marrow smear. Because of the presence of atypical lymphocytes on the peripheral blood smear and blast-like cells in the bone marrow, acute lymphoblastic leukemia was initially considered.

TABLE II: Hematological Findings

	SB	VB
WBC/mm^3	16.600* 32.000**	16.800* 3.500** 4.100***
LYMPHOCYTES (%)	60* (15% ATYPICAL) 98**	75* (10% ATYPICAL) 86** 90***
NEUTROPHILS (%)	30* 2**	22* 6** 10***
MONOCYTES (%)	10*	3* 8**
THROMBOCYTES	ABUNDANT* REDUCED**	ABUNDANT* ABUNDANT** REDUCED***
BONE MARROW	COVERED WITH LYMPHOCYTES (BLAST-LIKE CELLS WERE PRESENT	LYMPHOCYTOSIS* HYPOCELLULAR EARLY** MYELOID ELEMENTS WERE INCREASED
LIVER	ATYPICAL MONONUCLEAR CELL INFILTRATION IN PORTAL AREAS (POSTMORTEM LIVER BIOPSY)	

* When first seen
** Ten days after admission
*** Twenty days after admission

Immunological findings and Epstein-Barr virus (EBV) serology are shown in Tables III and IV. Except for anti-VCA IgM in Case 2, both patients failed to mount anti-EBV antibodies. EBV genome determination was performed only in Case 2, and was found to be positive in the lymph node. The mother had high anti-VCA and anti-EA titers (EBV serology was studied with the kind assistance of the late Dr. W. Henle and the EBV genome determinations were performed by Dr. G. Klein).

TABLE III: Immunological Findings

	SB	VB
E-rosettes	49	69
Delayed type Skin tests	NT	Positive with PHA, Candida, SK-SD
Serum Ig's (mg/dl)		
IgG	630	< 200
IgM	485	425
IgA	115	93
In vitro response to PHA (cpm STI/UNSTI)	13916/10219 Control 8441/119	6685/66 Control 4670/40

TABLE IV: EBV SEROLOGY

		SB	VB	MOTHER	FATHER
VCA	IgM	1/10	1/20	–	–
	IgA	1/10	–	–	–
	IgG	1/10	1/10	1/6 640	1/1 160
EA	D	1/10	1/10	1/10	1/10
	R	1/10	1/10	1/40	1/10
EBNA		1/2	1/2	1/20	1/10
EBV GENOME		NOT TESTED	POSITIVE (IN THE LYMPH NODE)		

Histopathological Findings

Only a postmortem liver biopsy could be taken in Case 1 which revealed atypical mononuclear cell infiltration in the portal areas. But a complete postmortem examination in Case 2 revealed the absence of corticomedullary demarcation and

Hassal's corpuscles in the thymus (Fig. 2). Lymphocytes were depleted and there was an increase in perilobular fibrous tissue. Both the lymph nodes and white pulp of the spleen lacked lymphoid follicles and germinal centers (Figs. 3, 4). Periarteriolar lymphoid sheats were depleted of lymphocytes in the spleen. Punched out-like ulcerations were noted in the intestinal tract, and scattered

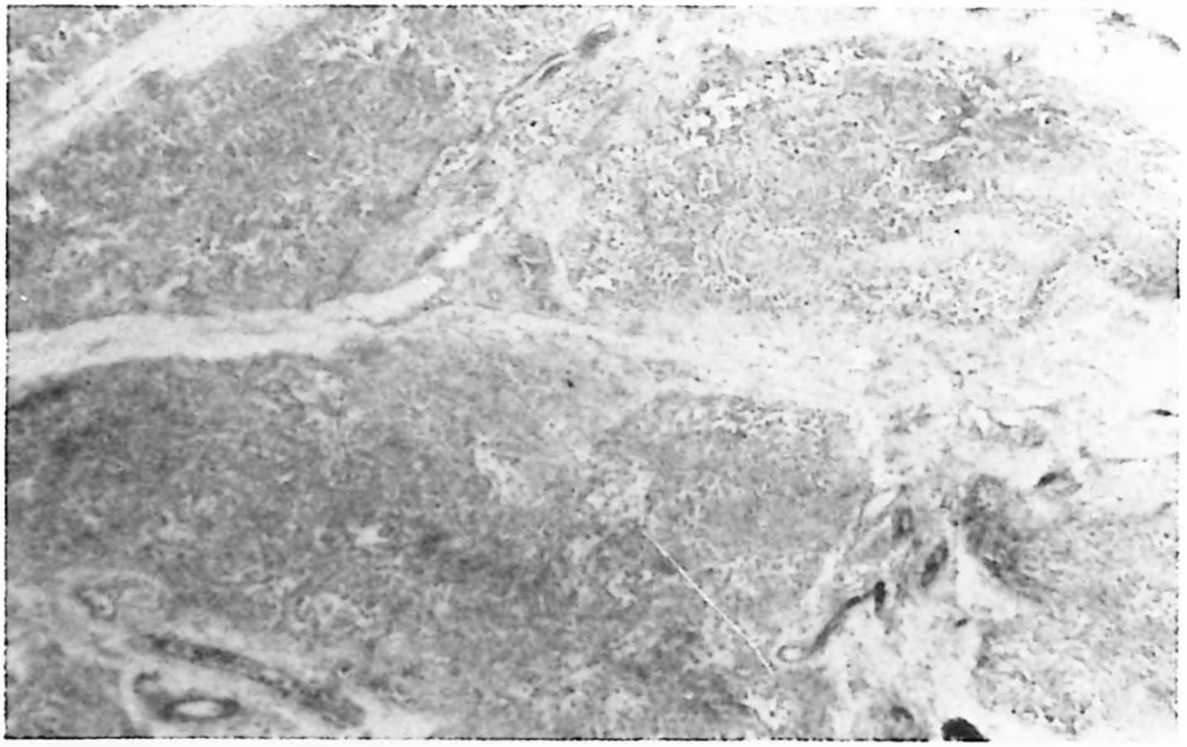

Fig. 2: Thymus showing absence of corticomedullary demarcation and Hassal's corpuscles.

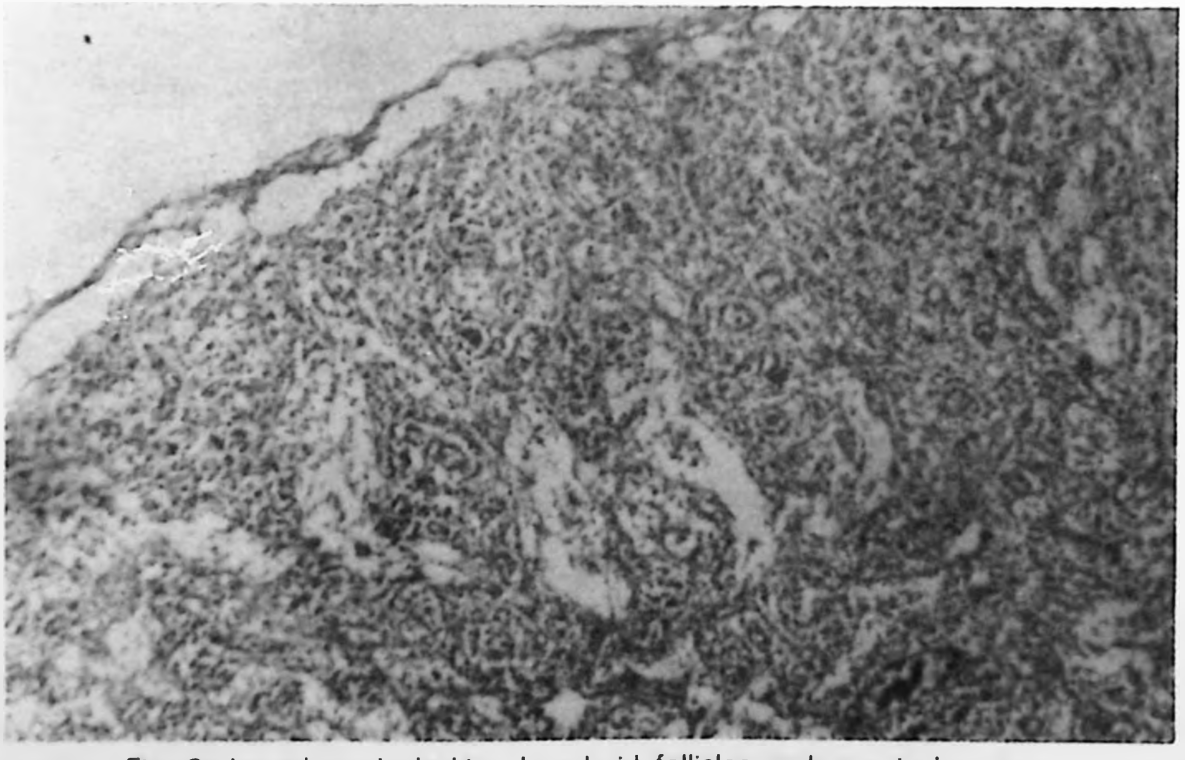

Fig. 3: Lymph node lacking lymphoid follicles and germinal centers.

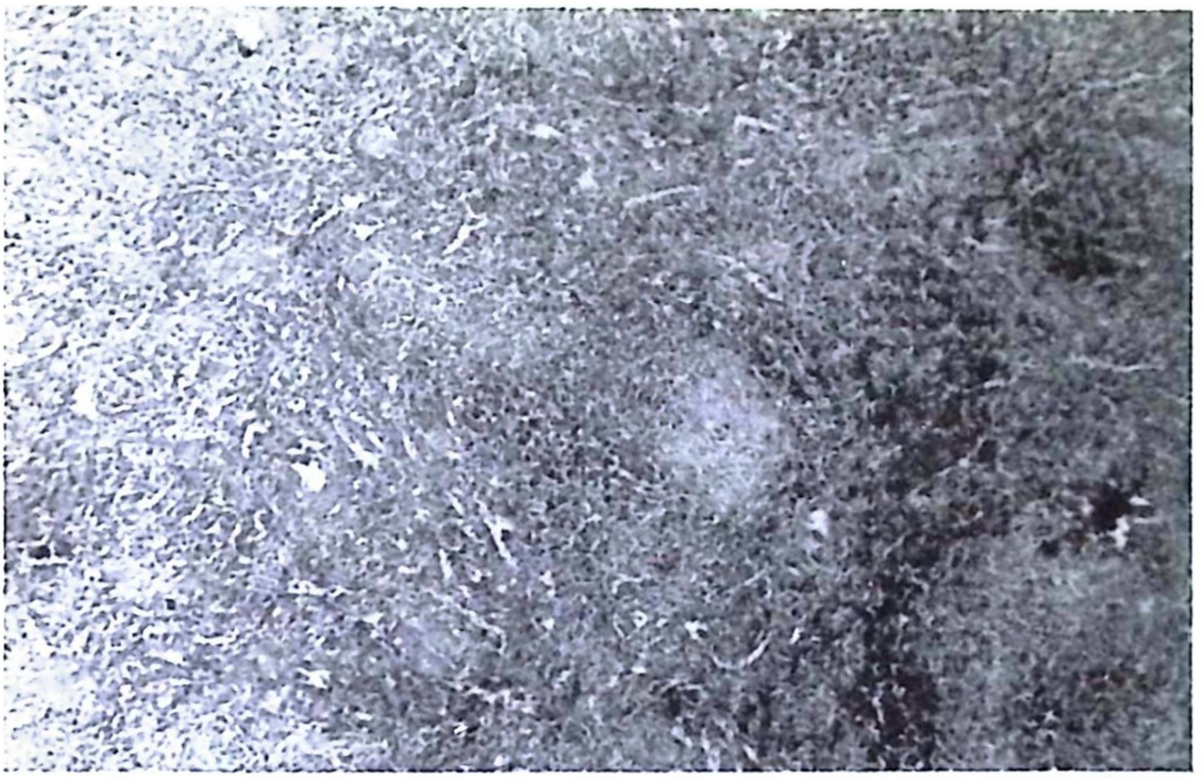

Fig. 4: Lack of lymphoid follicles in white pulp and depletion of lymphocytes in periarteriolar sheats in spleen.

plasma cells were noted in the submucosa (Fig. 5). There were no demonstrable Peyer's patches. The portal areas of the liver were infiltrated by histiocytes, plasmocytoid cells and lymphocytes (Fig. 6).

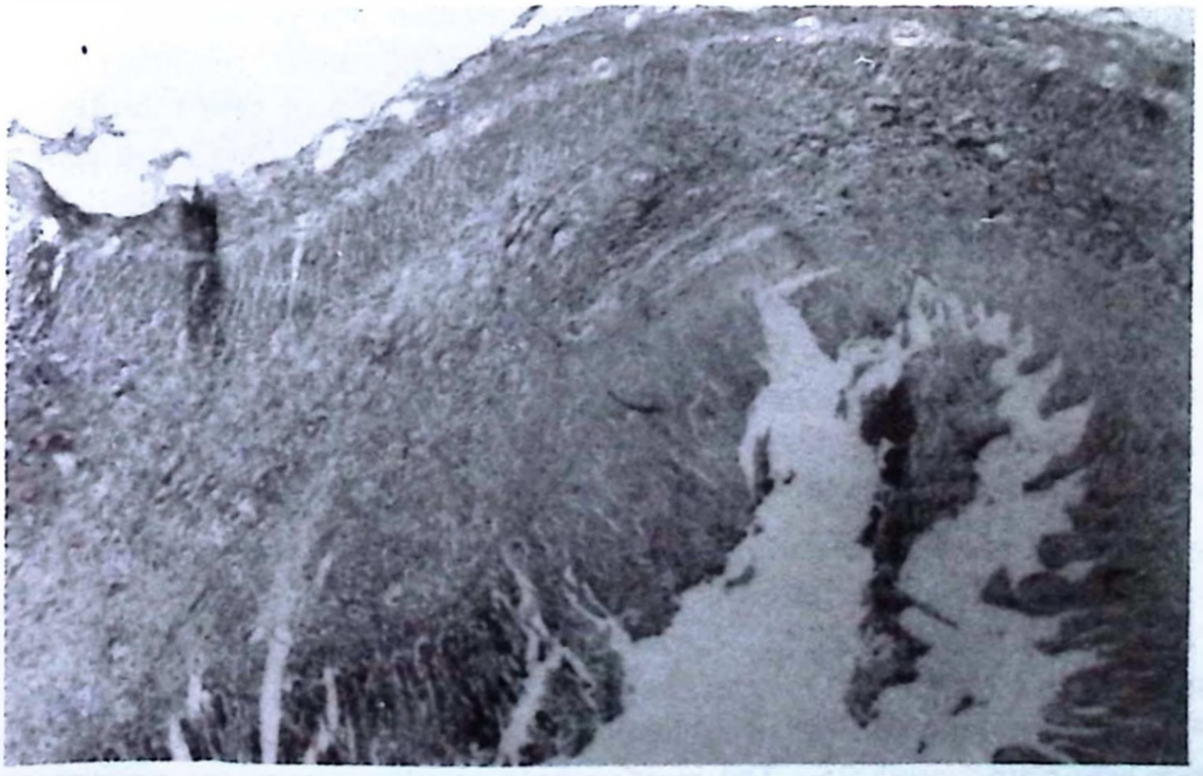

Fig. 5: Ulceration in intestinal tract and scattered plasma cells in submucosa; no demonstrable Peyer's patches.

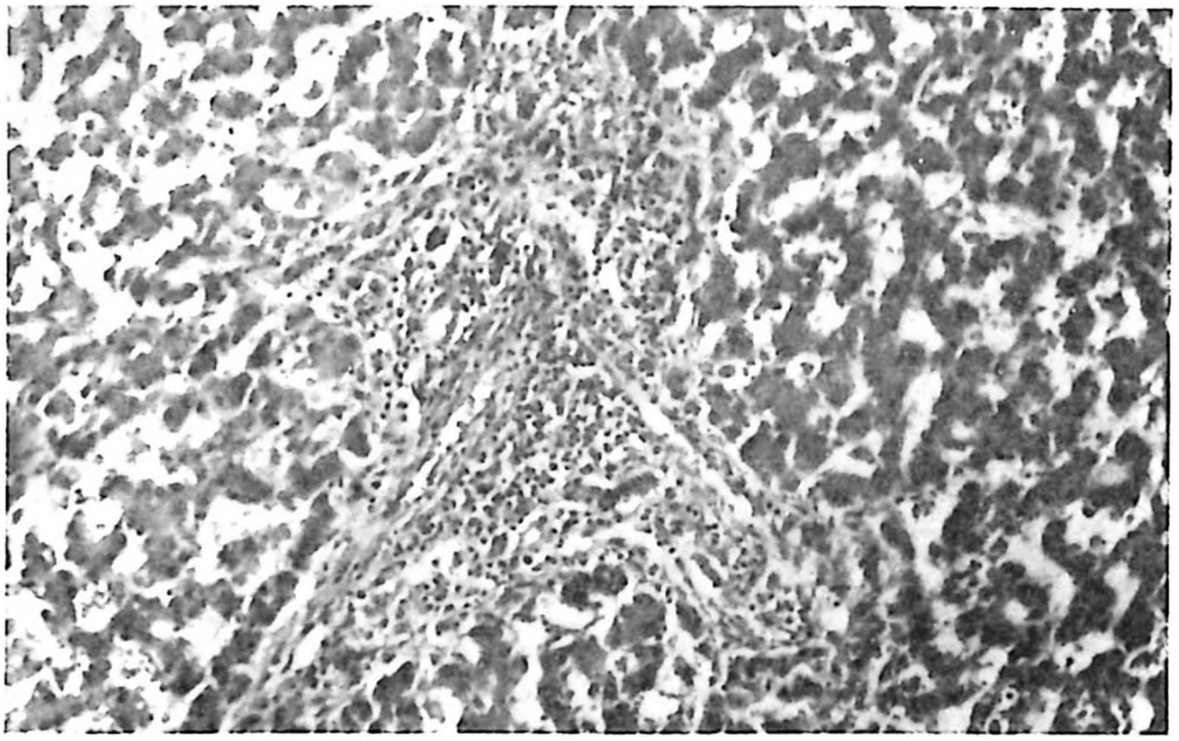

Fig. 6: Infiltration of portal areas by histiocytes, plasmocytoid cells and lymphocytes.

Discussion

We presented two brothers with fatal infectious mononucleosis. The age of onset, clinical course and duration of illness until demise, and the laboratory findings (except serum IgG levels) were similar in these two boys. They demonstrated the diagnostic criteria of XLP[4,2] (e.g., death resulting from a disease whose clinical picture indicated IM, the presence of EBV infection, the failure to mount anti-EBV antibodies, and a mother with both high anti-VCA and anti-EA titers). In addition, the maternal aunts and uncles in this family died of a disease that possibly had an infectious origin or was the result of a birth defect.

In Case 2 morphological alterations of the thymus as well as secondary lymphoid tissues mimicked those seen in patients with severe combined immunodeficiency disease (SCID). Destruction and depletion of the thymus gland have been noted in patients with XLP[2]. Thereafter, these patients develop marked depletion of thymus-dependent regions of the lymph nodes and spleen.

Mroczek et al[6] studied the thymic lesion in 35 XLP syndrome and sporadic fatal IM patients and observed six patterns: 1. Active lymphoproliferation with effacement of Hassal's bodies (HB), 2. Normal appearing HB (only three patients with XLP showed this pattern), however, calcified bodies were seen, precocious for age, 3. Moderate reduction of HB, 4. Marked depletion of HB, 5. Absence of HB, 6. Stress-associated involution. The histopathological findings of our patient were similar to pattern 5.

Possible mechanisms suggested for thymic epithelium destruction in XLP are: 1. Alteration of HLA antigens by viral infection which may lead to misdirected cytotoxic T cells that destroy the epithelium, 2. EBV may cause fusion of lymphoid cells with thymic epithelium leading to entry of virus into the epithelial cells; later epithelial cells undergo necrosis and disappear[2]. In fact, Seshi et al[7] have implanted EBV receptors into thymic epithelial cells by Sendai virus fusion thus creating a portal of entry for the virus. Cells die after several days in culture. It has been suggested that thymic hormones may restore the host immune response in patients with fatal EBV-induced lymphoproliferative disorders (provided that the thymus lesion is a common feature of all individuals)[2].

Recently, restriction fragment length polymorphism (RFLP) markers have been used to localize the XLP locus on the chromosome[8]. With the use of this new technology, it is now possible to predict which members of a family with XLP are carrier females and to diagnose the syndrome prenatally[8].

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LYMPHOSCINTIGRAPHIC EVALUATION OF PRIMARY CONGENITAL LYMPHEDEMA*

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Summary: Caner B, Günaydın E, Yalaz K, Varoğlu E, Naldöken S, Bernay İ, Bekdik C. (Department of Nuclear Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey). Lymphoscintigraphic evaluation of primary congenital lymphedema. Turk J Pediatr 32: 117-121, 1990.

A-four-year-old girl with primary nonfamilial congenital lymphedema was evaluated by lymphoscintigraphy using ^{99m}Tc-Dextran. It was demonstrated that there was no accumulation of radioactivity in the left inguinal and femoral lymph nodes and faint accumulation in the contralateral lymph node. It was concluded that lymphoscintigraphy is a safe, reliable, nonninvasive technique which is helpful in the evaluation of a patient with lymphedema, especially small children in whom conventional contrast lymphangiography offers some challenges. *Key words: primary lymphedema, lymphoscintigraphy, ^{99m}Tc-Dextran..*

Lymphedema is usually difficult to diagnose in childhood. Although contrast lymphangiography offers an excellent morphologic evaluation of the lymphatics, the surgical exposure of the lymphatics that is required may be difficult in an edematous extremity, especially in children, in whom general anesthesia is also necessary. Moreover, the contrast material itself may be irritating, and its administration can lead to pulmonary complications[1]. We present a case of bilateral primary lymphedema in the lower extremity who underwent lymphoscintigraphy, a safe, noninvasive, reliable method which does not require general anesthesia, skin incision, or lymphatic dissection[1].

Case Report

A four-year-old girl with swollen lower extremities extending from the inguinal area to the fingertips was admitted to the Hacettepe University Children's Hospital. A gradual onset of swelling of the left foot which extended to the inguinal area was noticed soon after she was one year old. The patient

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subsequently developed swelling of the right foot. There was gradual swelling of both lower extremities until the age of four, when she was brought to our Hospital (Fig. 1).

Her prenatal, natal and postnatal histories were unremarkable, and there was no history of trauma, infection or familial occurrence of such lower limb asymmetry.

Physical examination on admission revealed a weight of 18.5 kg, height 102 cm, temperature 36° C, heart rate 168 /min, and blood pressure 120/90 mm Hg. Both lower extremities were grossly edematous. There was no pain or color change of the skin. The involvement of the right thigh was less than that of the left. The inguinal lymph nodes were not palpable. Microlymphadenopathy of the right mandibular and jugular were noticed. Laboratory studies revealed a hemoglobin of 12.8 g/dl, hemocrit 35 %, and white blood cell count 6000/mm^3.

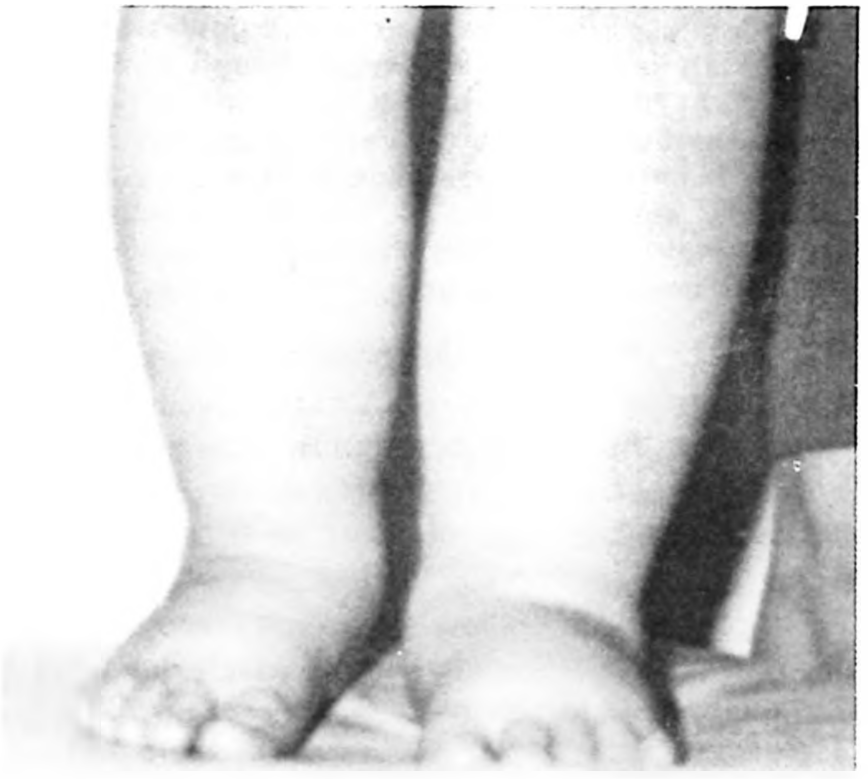

Fig. 1: Photograph of a four-year-old girl with long standing primary lymphedema of the lower extremities.

Since the patient developed a urinary tract infection following admission to the hospital, anesthesia required for contrast lymphangiography could not be given, and consequently, lymphoscintigraphy was performed. 150 µ Ci ^{99m}Tc-Dextran in a volume of 0.05 ml prepared in a previously described manner, was injected intradermally in the first web space of each foot[2]. Scintigrams were taken one, two, three and six hours postinjection, using a large field, all purpose, low energy collimator (Siemens ZLC II). Both legs and lower abdomen including the liver area

Fig. 2: Normal appearance of iliopelvic lymphoscintigraphy. Note intense uptake of inguinal lymph nodes.

Fig. 3: Iliopelvic scintigraphy of patient taken at the sixth hour after bilateral interdigital injection. There is diminished inguinal node accumulation on the right side, whereas, almost no accumulation on the contralateral side (arrows). Note markedly diminished visualization of the liver.

were imaged. Although the scintigrams failed to demonstrate the left inguinal lymph nodes, a faint visualization of the right inguinal lymph nodes was noted one hour postinjection. The external iliac lymph nodes could not be visualized. There was no remarkable change up to six hours postinjection. Almost all the radioactivity remained unchanged on both injection sites (Figs. 2, 3).

In light of all the clinical and laboratory findings, the patient was diagnosed as having primary nonfamilial congenital lymphedema. She was referred to the Department of Cardiovascular Surgery for a possible lympho-venous shunt operation.

Discussion

Primary lymphedema has been classified on the basis of age of onset as: congenital, lymphedema praecox (prior to age 35) and lymphedema tarde (after age 35). Lymphedema is also subdivided into familial and nonfamilial forms. The familial form is known as Milroy's disease, probably an autosomal dominant disorder[3-5]. Congenital lymphedema has been reported in Turner's syndrome (gonadal dysgenesis). A nonfamilial form of lymphedema has also been described. Letessier-Meige disease, a hereditary form, is the later onset form of primary lymphedema[5]. Since there was no familial history of this disease in our case, and the onset occurred at a very early age, the diagnosis of the nonfamilial congenital form was considered.

In order to establish the diagnosis of primary lymphedema it is essential to demonstrate the lymphatic pathways. For this purpose, conventional contrast lymphangiography is the classical imaging modality.

Although contrast lymphangiography can provide much better anatomic delineation of lymphatic channels and nodes, the lymphatic cannulation which is required is usually difficult in an edematous foot. Besides, it is known that lymphangiographic contrast medium may cause further inflammation of already diseased lymphatic vessels[1].

Lymphoscintigraphy plays an especially important role in diagnosing primary lymphedema in children, as was demonstrated in our case. ^{99m}Tc-Dextran is a known radiopharmaceutical for lymphoscintigraphy[6]. ^{99m}Tc-Dextran injected intradermally enters the lymphatic system and is deposited in lymph nodes along the route. After a node is saturated, the radioactivity passes onto further nodes. Some radioactivity bypasses all the nodes, and enters the thoracic duct resulting in visualization of the liver. Poor visualization, cut-off lymphatic channels and/or poor nodal uptake indicate the presence of hypoplastic or aplastic channels, as in our case. The absence of radioactivity in the liver is also a supportive finding.

In our case lymphoscintigraphy performed up to six hours postinjection showed almost a complete absence of radioactivity in the left inguinal lymph node groups, faint uptake in the right inguinal lymph node chain, and almost no visualization of the liver, indicating aplastic lymphatic channels on the left side and hypoplastic channels on the right side. These findings correlated well with the history of the patient, which revealed that the onset of swelling of the left foot preceded that of the right and that the involvement was to a less of a degree. According to the scintigraphic findings, the patient was diagnosed as having primary nonfamilial congenital lymphedema.

In conclusion, lymphoscintigraphy should be the first choice of imaging modality in the evaluation of the patient with lymphedema, children in particular.

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The Turkish Journal of Pediatrics 32:123-126, 1990

TRAUMATIC ESOPHAGEAL PERFORATION IN A PREMATURE INFANT*

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*Melih Bulut MD****

Summary: Tekant G, Abbasoğlu L, Sever N, Bulut M. (Department of Pediatric Surgery, Şişli Children's Hospital, Istanbul, Turkey). Traumatic esophageal perforation in a premature infant. Turk J Pediatr 32: 123-126, 1990.

We presented a case of neonatal hypopharyngeal perforation resulting from extraction of a breech presentation, with symptoms of regurgitation at all feedings and excessive salivation. Inability to pass a nasogastric tube suggested the diagnosis of esophageal atresia. The diagnosis and treatment of this condition is discussed, and the literature is reviewed. *Key words: neonate, esophagus, hypopharynx perforation.*

Traumatic perforation of the hypopharynx or cervical esophagus was first reported by Eklöf et al[1] in 1969. This condition may occur during or just after delivery, and may present with symptoms of esophageal atresia in a newborn infant. The physician may be unable to pass a nasogastric tube[1-5]. This article presents a case of hypopharyngeal perforation, its diagnosis and treatment, and a review of the literature.

Case Report

A two-day-old male infant who was born prematurely after 36 weeks' gestation and weighed 1600 g was admitted to the pediatric surgery clinic of the Şişli Children's Hospital, with serosanguineous oral drainage and inability to feed. The prenatal history was normal. Delivery was a breech presentation where the obstetrician placed her finger in the infant's mouth to assist in the birth At birth, the infant had mild cyanosis and respiratory distress. He had a one minute Apgar score of six. With no medical intervention, the infant's general condition improved, and the Apgar rating was upgraded to eight at five minutes. The patient was transferred to the premature care unit because of his low birth weight.

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At admission, the patient vomited his first feeding and constantly regurgitated saliva. Ronchi were also heard over the lungs. An attempt to pass a nasogastric tube failed, and the patient was transferred to the pediatric surgery clinic, with an initial diagnosis of esophageal atresia.

During fluoroscopy, the catheter could not be passed from the nose into the stomach, being arrested in the cervical region. Barium was instilled through the catheter, and x-rays revealed contrast material passing into the stomach (Fig. 1). Contrast material from the upper end of the esophagus diverted into a narrow tract, closely following the esophagus posteriorly and reached the diaphragm.

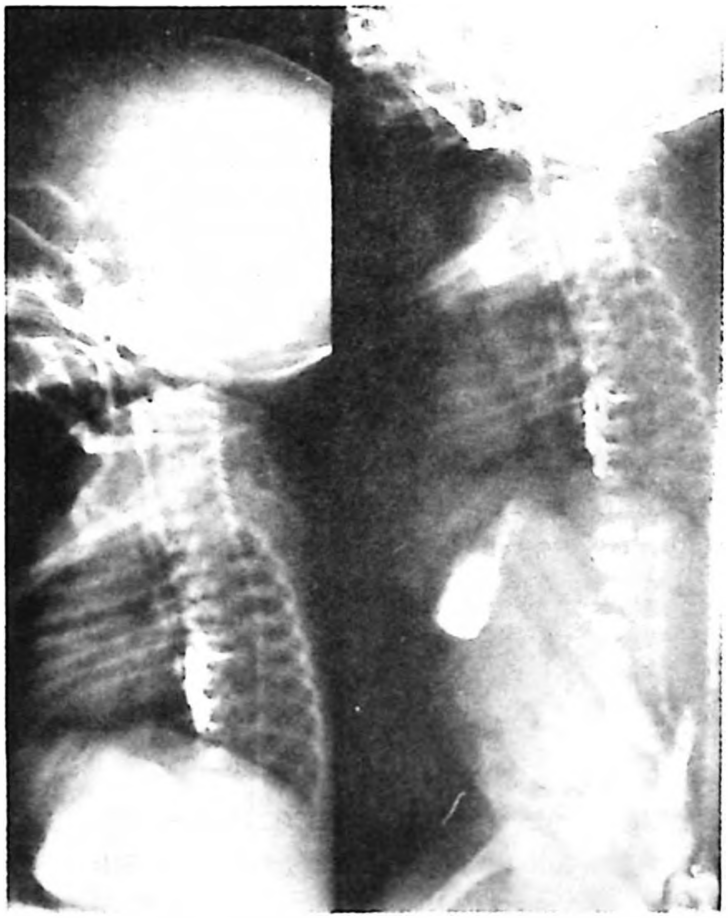

Fig. 1: Contrast material seen in the stomach and the posterior mediastinum.

Esophagoscopy revealed a semilunar tear in the posterior wall of the esophagus just above the cricopharyngeal narrowing. A diagnosis of traumatic esophageal perforation was made and the oral feedings were immediately discontinued. Total parenteral nutrition (TPN), systemic antibiotics and cimetidine were administered for ten days along with repeated oropharyngeal aspirations till the infant's regurgitation of secretions decreased and swallowing started. An 8 F catheter was passed into the stomach under endoscopy and alimentation was continued with a nasogastric tube for ten days. Repeat esophagoscopy showed healing to

be complete and oral feedings were started. Chest x-rays taken twenty-five days after admission were normal except for the barium remnants detected in the posterior mediastinum (Fig. 1).

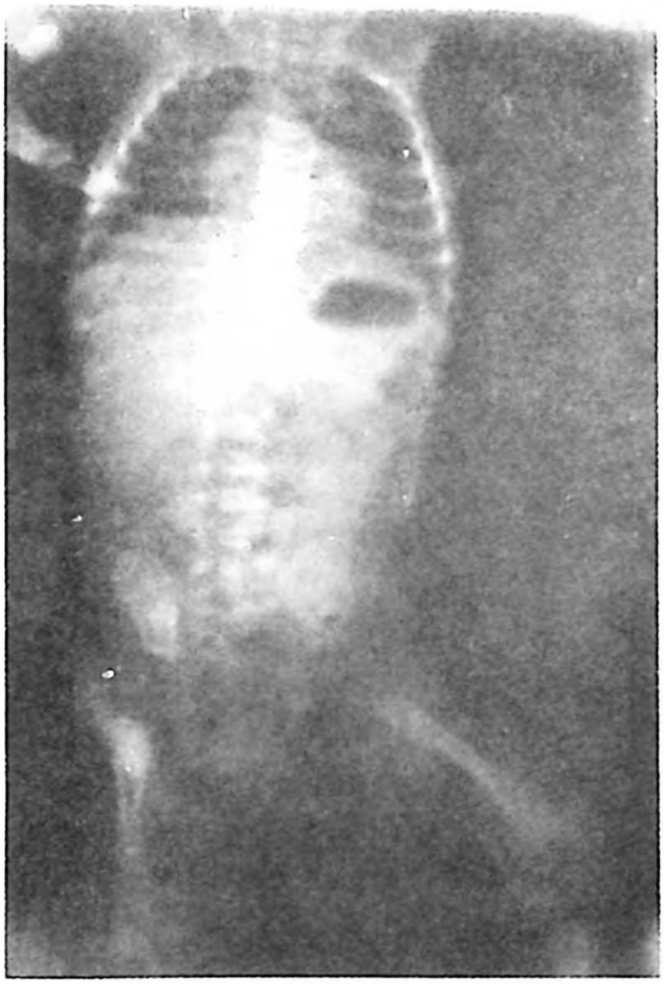

Fig. 2: Remnants of contrast material still seen in the posterior mediastinum.

Discussion

Cricopharyngeal perforation during the newborn period may result from traumatic maneuvers, such as the placement of an endotracheal or nasogastric tube or delivery of a breech presentation[1-5]. Routine use of suction catheters in delivery wards, especially in low birth-weight infants with the baby's neck in an extended position, may lead to trauma of the cervical esophagus[5]. Besides, to assist in the delivery of a baby during a breech presentation, the obstetrician places his finger in the mouth of the newborn. Forceful manipulation may easily perforate the fragile hypopharynx of the neonate. If symptoms of excessive salivation, choking and cyanosis are present in a neonate that has been vigorously manipulated, rupture of the cervical esophagus must be considered[3-5]. In such event, the perforation is a result of the obstetrician's act during extraction of the infant.

Infants with this injury present with signs of severe esophageal obstruction, with inability to feed, excessive salivation, and cyanosis. This condition has been attributed by Girday et al[2] to be a spasm of the cricopharyngeus.

Inability to pass a nasogastric tube when these signs are present leads to an initial diagnosis of esophageal atresia. A definite diagnosis is made by radiographic and endoscopic investigation. Radiologic studies demonstrate the tract lying posterior to the esophagus, while endoscopy can identify a tear in the cervical esophagus[1-4].

Differential diagnosis includes congenital esophageal diverticula, esophageal duplication, esophageal atresia, and laryngotracheoesophageal cleft[4]. Proper interpretation of radiographs and endoscopy will assure correct evaluation and provide proper therapy for these infants.

Since none of the reported patients with perforation of the cervical esophagus present with life-threatening mediastinitis, pneumomediastinum or pneumothorax, the choice of therapy is conservative. It consists of the administration of TPN, systemic antibiotics and oropharyngeal aspiration[3,4]. Total parenteral nutrition is life-saving therapy for these children, who are unable to receive oral feedings for at least 10 to 14 days, till the esophagus is completely healed. Our patient received TPN for ten days, with no serious metabolic complication, till nasogastric feeding could be tolerated. If TPN cannot be administered, a feeding gastrostomy may be performed.

In this situation, immediate surgery is not desired because the esophagus heals spontaneously. Surgical intervention should be reserved for complications of mediastinal or cervical abscess, pneumomediastinum and pneumothorax This situation contrasts with the adult, who generally requires drainage and repair of the perforation[3,4].

As a result, early diagnosis and correct medical therapy is sufficient to avoid the stress of surgery in these already non-tolerant premature infants.

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IDIOPATHIC LONG Q–T SYNDROME*

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Summary: Özer S, Çeliker A, Oto A, Özme Ş. (Hacettepe University Institute of Child Health, Ankara, Turkey). Idiopathic long Q-T syndrome: a case report. Turk J Pediatr 32: 127-133, 1990.

The association of Q-T interval prolongation, syncope and sudden death is known as the long Q-T syndrome. The syndrome may be familial, associated with congenital deafness, or idiopathic. The diagnosis is based on the electrocardiographic finding of a prolonged Q-T interval with or without T wave abnormalities. In this article, we present a nine-year-old boy admitted to the Hacettepe University Children's Hospital with complaints of syncopal episodes. The prolonged Q-T syndrome was diagnosed as the cause of the syncopal attacks. In addition, the same syndrome was detected in his father. The prolonged Q-T syndrome should be considered in the differential diagnosis of cases with recurrent, unexplained syncope in the pediatric age group. *Key words: Idiopathic long Q-T syndrome, syncopal attacks, family history.*

Idiopathic long Q-T syndrome is a hereditary condition with a fatal outcome[1,2]. The diagnosis of this syndrome is based on the electrocardiographic finding of a prolonged Q-T interval[1,3,4]. The treatment of choice for preventing syncopal attacks in this syndrome is either the administration of beta-adrenergic blocking agents or performing a left stellectomy[3,5,6]. In this article, we present a case who was admitted to the Hacettepe University Children's Hospital with ventricular tachycardia and syncope. The diagnosis of idiopathic long Q-T syndrome was made by electrophysiologic ambulatory Holter monitoring studies.

Case Report

A nine-year-old boy was admitted to the Hacettepe University Children's Hospital with complaints of syncopal attacks which were associated with urinary incontinence. However, there was no tonic-clonic movement or excessive salivation. About three years ago, he came to the Hacettepe University Children's

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Hospital with a history of fainting. He was then diagnosed as having epilepsy and treated with antiepileptic drugs but this therapy did not prevent the syncopal attacks. In addition, the attacks were induced by exercise and stress.
His family history revealed that his grandmother and grandfather were cousins. The son of the patient's uncle died at the age of fifteen following an epileptic seizure, and the patient's brother, died at age 12 at the Hacettepe University Children's Hospital, where he was under treatment for epilepsy.
His father suffered from palpitation and syncopal attacks induced by exercise but he did not take any drugs for these complaints (Fig. 1).

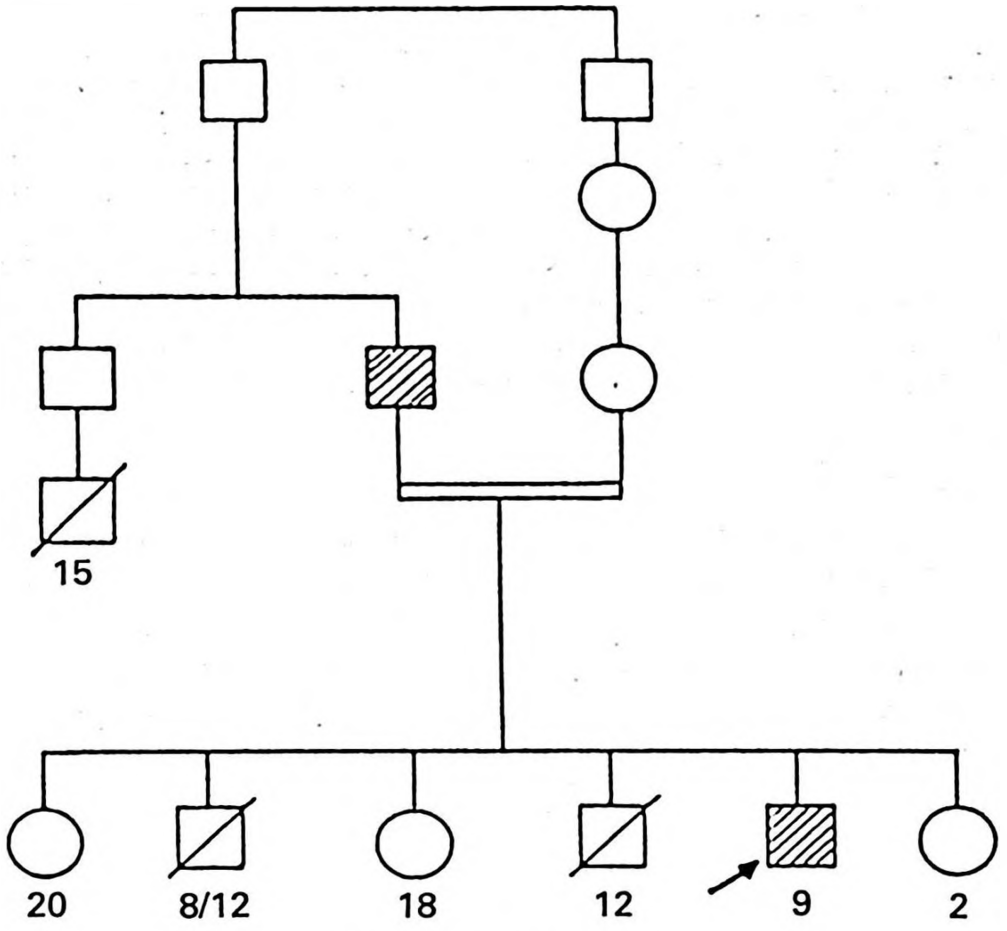

Fig 1: Family pedigree.

Physical examination at admission revealed that the boy's weight was 25 kg, height 133 cm, and arterial blood pressure 120/80 mm Hg. On auscultation the heart rate was regular at 80 per minute and there was no significant murmur. Laboratory studies revealed a hemoglobin of 13.65 g/dl, white blood cells 4200 per/mm^3, and plasma calcium 9.6 mg/dl. The electrocardiogram revealed that the sinus rhythm and the QRS mean vector was +15° in the frontal plane, PR 0.16 sec, and the corrected Q-T interval was 0.42 sec (Fig. 2). Two-dimensional echocardiographic examinations showed normal left ventricular function and

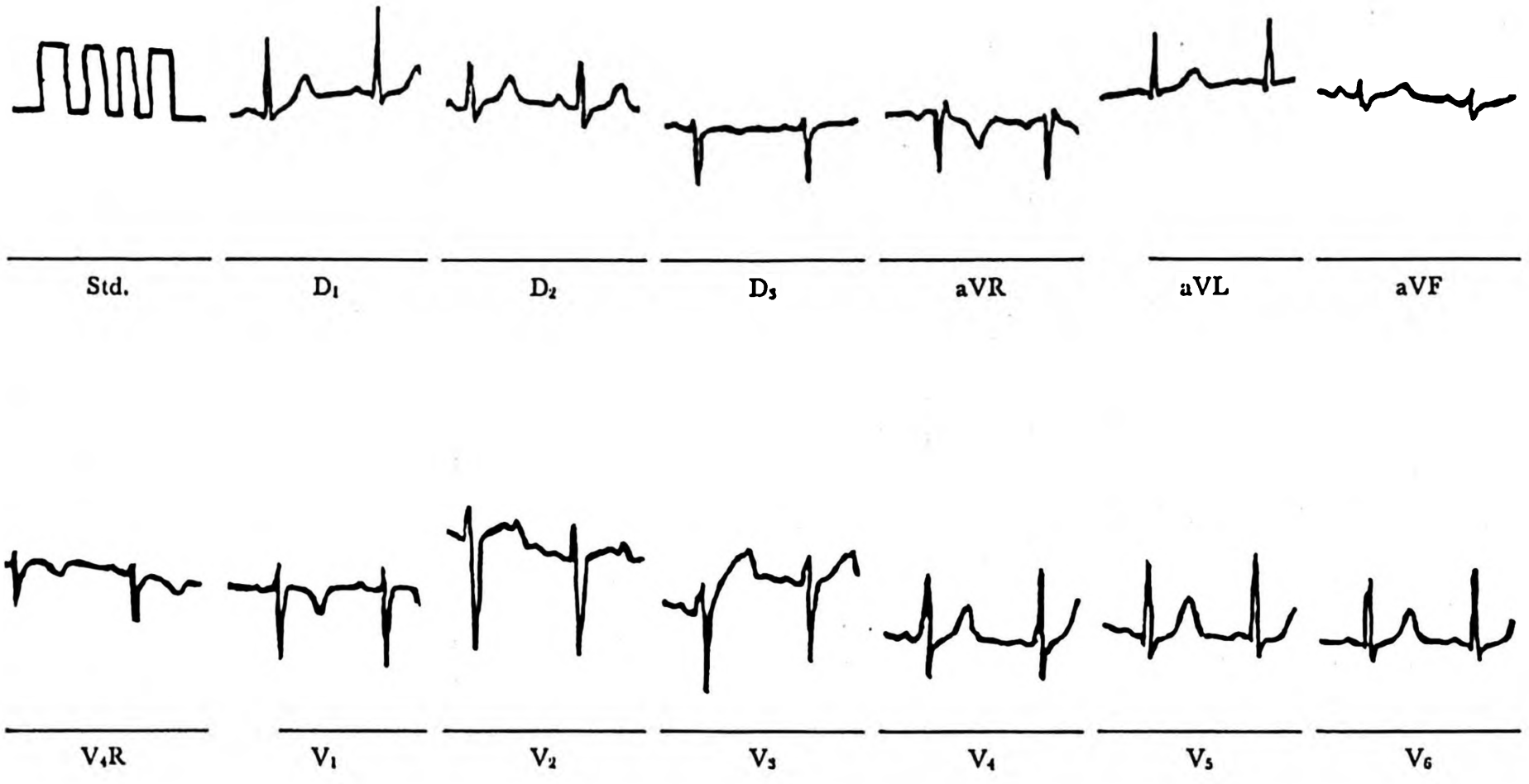

Fig 2: Electrocardiogram of the patient.

neither cardiomyopathy nor mitral valve prolapse was demonstrated. Pindolol (Visken^{R}) 2.5 mg/day was used to treat the tachycardia. After discontinuing the drug for one day electrophysiological studies were performed. Following local anesthesia, both femoral veins were catheterized and multipolar electrodes were placed in the high right atrium, low right atrium, His and right ventricular positions. The study was carried out according to a strict protocol, and Q-T interval 360 msec and corrected Q-T interval of 430 msec were measured respectively. The other parameters (RR, PR, QRS, QT, AH, HV, maximum sinus node recovery time, maximum corrected sinus recovery time, sinoatrial conduction time, Wenckebach point, effective atrial refractory period, effective atrioventricular node refractory period, effective ventricular refractory period) were normal, and despite several attempts, ventricular tachycardia could not be produced. A 24-hour ambulatory Holter-monitor study did not reveal any ventricular tachycardia (Fig. 3). Beta-adrenergic blockade as the choice of treatment in this case was very beneficial. After treatment with 2.5 mg pindolol, no syncopal attacks occurred.

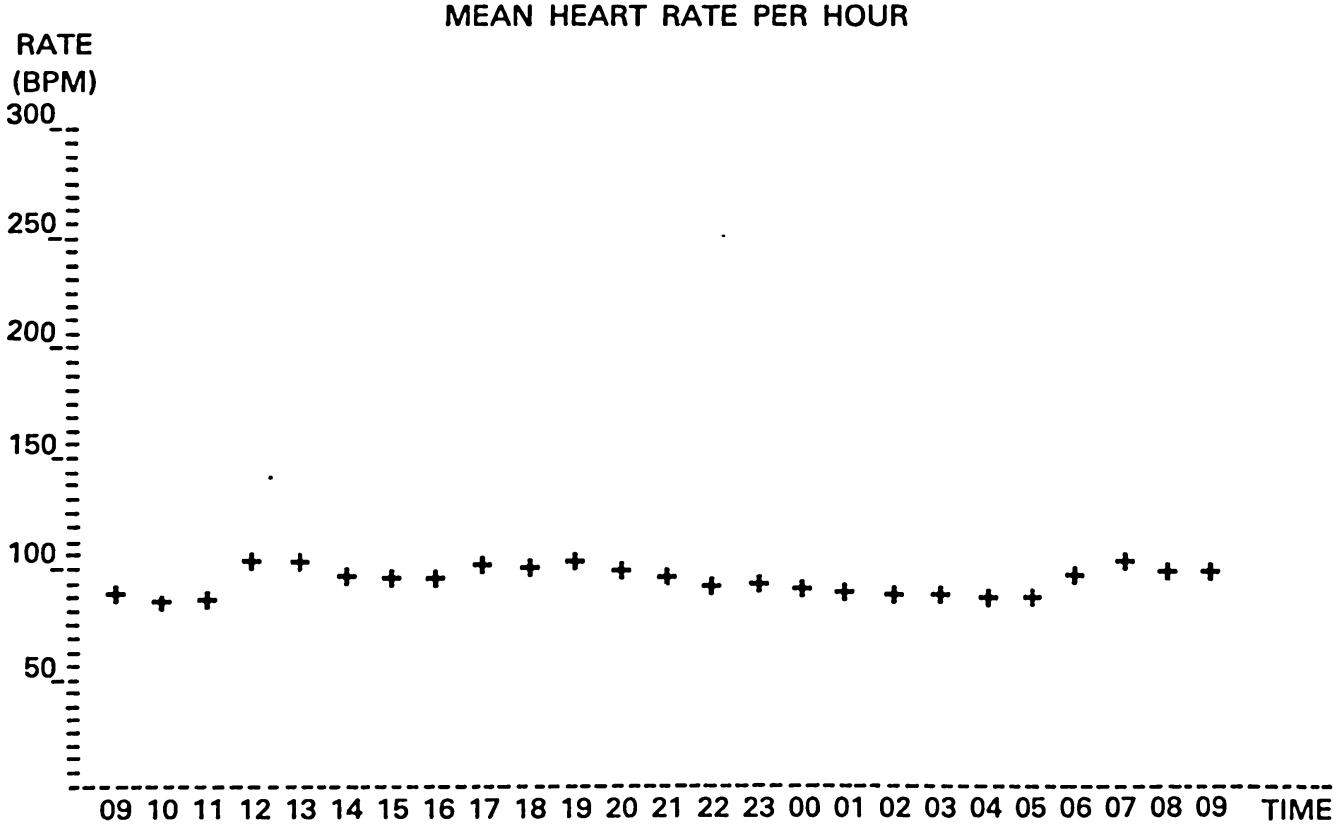

Fig 3: Mean heart rate per hour on the ambulatory Holter monitor.

The electrocardiograms of the patient's family members were evaluated for this syndrome, and a Q-T interval of 0.40 sec and a corrected Q-T interval of 0.43 sec were detected on the father's electrocardiogram (Fig. 4). Because of this finding, we ascertained that the father also had the idiopathic long Q-T syndrome.

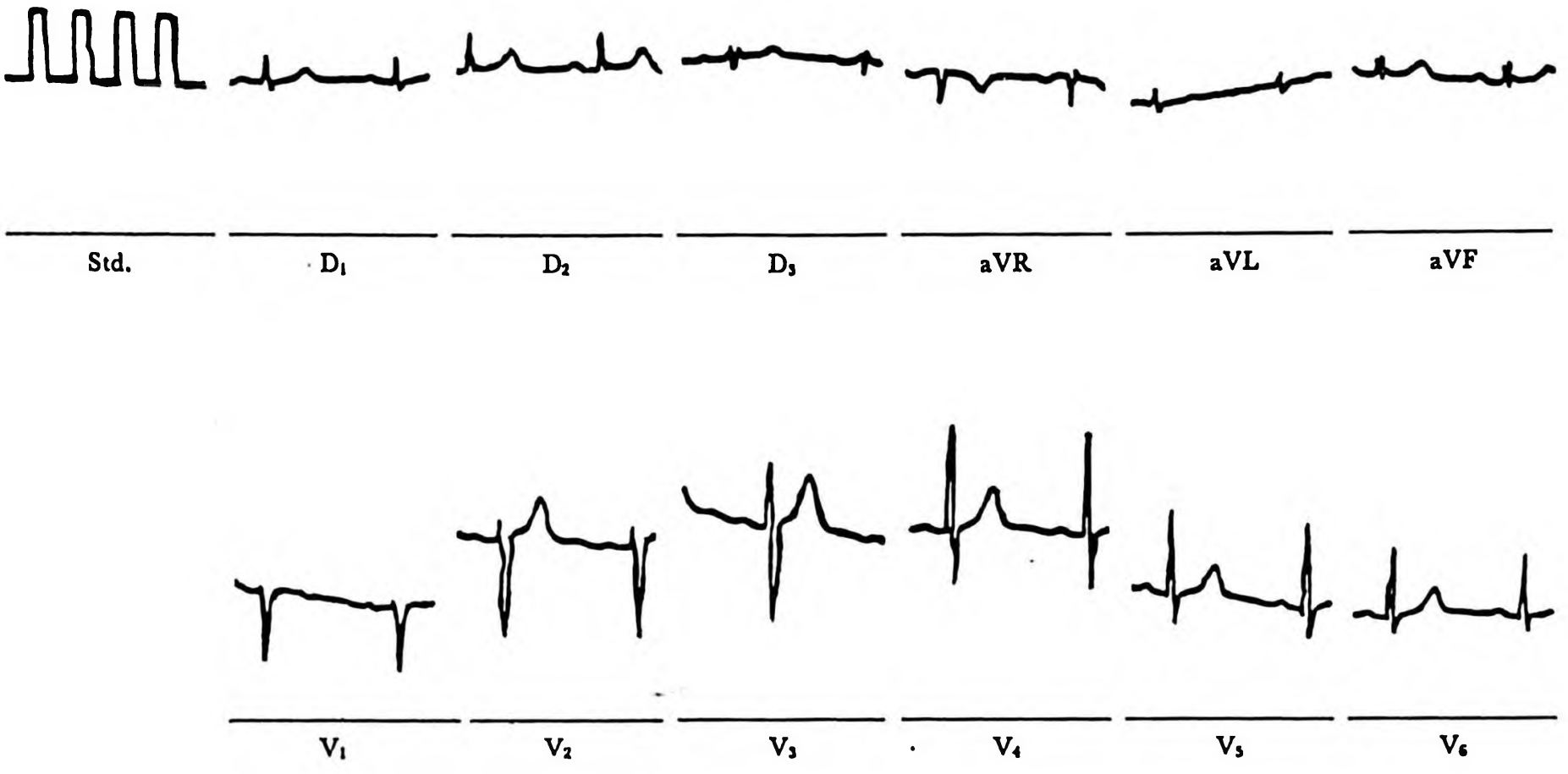

Fig 4: The father's electrocardiogram.

Discussion

Idiopathic long Q-T syndrome is a ventricular repolarization abnormality characterized by syncopal attacks and sudden death[1,2]. Some authors have investigated the characteristics of this syndrome[2,3], and Schwartz et al[1] have described the complete syndrome.

The Jervell and Lange-Nielsen syndrome is accompanied by congenital deafness and has an autosomal recessive pattern of inheritance. In the Romano-Ward syndrome, hearing is normal and an autosomal dominant pattern of inheritance has been confirmed[1,2]. A recent report suggests a probable close linkage between the HLA A 9 BW 54 and the Romano-Ward syndrome[4,7].

The Q-T interval varies with the heart rate. The corrected Q-T interval can be found by using Bazett's formula[4,7] given below:

$$QTc = \frac{QT}{\sqrt{R\text{-}R}}$$

The corrected Q-T interval is abnormal if it is prolonged beyond 0.40 sec in children under 12 years of age or is greater than 0.43 sec in children 12 years of age and over[4]. The affected individuals have ventricular tachycardia and syncope induced by exercise and stress[1,3,5].

Schwartz proposed some diagnostic criteria for this syndrome[1] (Table I). The diagnosis of idiopathic long Q-T syndrome should be made in the presence of two major criteria or one major and two minor criteria[1]. Bhandari et al[8] studied 15 patients with the idiopathic long Q-T syndrome with a thorough electrophysiologic protocol, and concluded that electrophysiologic studies were useless.

TABLE I. Diagnostic Criteria

Major	Minor
– Prolonged Q-T interval (QTC > 440 msec)	– Congenital deafness
– Stress-induced syncope	– Episodes of T wave alternans
– Family members with long Q-T syndrome	– Low heart rate (in children)
	– Abnormal ventricular repolarization

When a patient with the idiopathic long Q-T syndrome is identified, treatment with beta blockers should be instituted immediately. If the syncopal episodes continue despite full-dose beta-adrenergic blockade, a high thoracic left sympathectomy should be performed[1,3,5,6]. With adequate treatment, a reduction in the occurrence of syncope and sudden death has been achieved[3,5].

The clinical and electrocardiographic studies of both our patient and his father confirmed the diagnosis of the idiopathic long Q-T syndrome. In addition, we suggest that the patient's brother, who had suffered from syncopal attacks, may have died from the same syndrome.

Beta-blocker therapy was instituted in our case, and as a result, the syncopal attacks did not occur. We are planning a high thoracic left sympathectomy if tolerance develops.

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CELL CYCLE ANALYSIS OF NORMAL AND MALIGNANT LYMPHOID CELLS*

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Various stages of lymphoid differentiation in the bone marrow (BM) and in the thymus are defined with combinations of monoclonal antibodies (McAbs) and heterologous antisera. The analysis of the proliferative capacity of these cell types can also be performed. One method is the separation of subsets of cells by fluorescent activated cell sorting or panning techniques followed by ^{3}H–thymidine incorporation and cell cycle analysis using propidium iodide[1]. Recently, the availability of McAbs to 5'bromo-2'deoxyuridine (BrdU), a thymidine analogue[2], and to Ki67, a nuclear antigen expressed by cells in cycle but not by cells in Go[3], has facilitated the immunological characterization of dividing cells. Double colour immunohistochemistry and double colour immunofluorescence methods including these Abs have been reported[4,5].

We have studied the proliferating capacity of immature lymphoid cells using multiple marker analysis. Our aim has been to combine the identification of surface, cytoplasmic and nuclear antigens with staining using BrdU and Ki67 McAbs. For this purpose cytocentrifuge preparations are fixed in cold methanol for 30 minutes, followed by immersion in $1x10^{-2}$ N NaOH for 10 seconds and in borate buffer for 5–10 seconds. Fixation in methanol alone, without immersion in NaOH, is optimal for Ki67 staining. With these methods, BrdU and Ki67 staining can now be included in triple colour labelling using specific second layers conjugated to fluorescein isothyocyanate (FITC), tetramethyl rhodamine (TRITC) and colloidal gold[6].

Phenotypic expression and proliferative activity during BM B cell differentiation

The proliferative activity and the differentiation process in the central lymphoid organs of mice have been extensively studied. The BM is a major source of new lymphocytes, and pre-B cells generate a number of small lymphocytes in the order of 10^{8} cells/day. Large cytoplasmic μ^{+}, surface μ^{-} ($c\mu^{+}$, $s\mu^{-}$) cells are the major proliferative compartment in the early stages of B cell differentiation[7,8] reviewed in 9.

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In man, the phenotypic changes of lymphoid populations in the BM have also been described. In human fetal BM, around the 17th gestational week, IgM^+ B cells represent 20-25% of all nucleated cells[10]. Relatively high percentages of early B cells express nuclear terminal deoxynucleotidyl transferase (TdT) and/or cμ in fetal and infant as compared to normal adult BM[10-13].

Early studies of the antigenic expression of B cell precursors in the human BM performed with heterologous antisera showed that TdT^+ cells are $HLA\text{-}DR^+$ and express the cALL antigen[14]. These findings have been confirmed and extended with other McAbs against B cell associated antigens. The conclusions of these investigations are as follows: Firstly, phenotypical differences can be found between B cells in the BM and in the peripheral lymphoid organs. On one hand, some antigens, like those identified by CD19[15] and anti Class II reagents, are expressed by virtually all B cells until they become plasma cells. On the other hand, some antigens are associated with particular stages of differentiation. Secondly, there is an orderly appearance of nuclear, cytoplasmic and surface antigens during B cell differentiation. Finally, leukaemic cells of the B lineage reproduce the phenotypic features found on the equivalent normal cells.

TdT^+ cells are Class II^+ and express proliferation-activation related structures such as transferrin receptors and Ca^{++} channels[11,12,16]. Additionally, they express a number of molecules whose function has not yet been fully elucidated. These are the antigens identified by CD10, CD19, CD24 and CD9 antibodies. While CD24 and CD9 are also expressed by non-B cells, CD19 appears to be exclusively B cell associated. CD10 is also found on non-B cells such as granulocytes and some T acute lymphoblastic leukaemia (T-ALL) blasts. Normal BM TdT^+ cells do not express IL2 receptors, but these can be induced by stimulation with phorbol esters (Campana D, unpublished observations).

The phenotype of pre-B cells, identified by the presence of cμ, represents a step forward in the B cell differentiation pathway[17,18]. Nuclear TdT is expressed only by a minority of these cells[12]. The initiation of the synthesis of structures for antigen recognition and the detection of these molecules in the cytoplasm of B cell precursors precedes the expression of surface CD20 and human leucocyte function associated (HLFA) antigens[19-21].

The pattern of early assembly followed by the surface insertion is not only found for Ig expression but also for the structures identified by CD22 McAbs. These molecules are present in the cytoplasm first and absent on the surface of TdT^+ and pre B cells. Subsequently at a later stage of differentiation CD22 is expressed on the B cell membrane[19].

The transition from pre-B cells to $sIgM^+$ lymphocytes is characterized by the progressive acquisition of CD37 and unclustered surface antigens such as RFB7 and Y29/55[19,20,22]. However, the complete set of surface antigens found on

mature B cells in peripheral lymphoid organs is not yet exhibited on these cells, and a number of structures such as the C3d receptor (CD21) and CD22 are absent. Recently, a functional role for some of these B cell associated molecules has been postulated. McAbs of the CD19, CD20 and CD22 clusters have been implicated in the control of B cell proliferation after stimulation with anti-IgM McAbs[23-25]. Complement receptors have also been indicated as important structures in the proliferation of B cells after antigen stimulation. Thus, the 'incomplete' phenotype of IgM^+, IgD^- B cells is in line with their functional 'immaturity'[26].

We have recently documented the proliferative activity of these different subpopulations of B cell precursors in man. We studied the incorporation of BrdU and the expression of nuclear Ki67 of B cell precursors in adult as well as infant and regenerating BM. In order to precisely identify cell subsets, combinations including BrdU or Ki67 with nuclear TdT, surface CD10, cytoplasmic IgM and surface RFB7 (CD20-like) were used.

The results of these studies indicate that during B cell ontogeny in the BM the major proliferative compartments are represented by TdT^+ cells and $c\mu^+$ pre-B cells. About 40 % of TdT^+ cells express Ki67 and approximately 25 % were synthetising DNA at any given time. An even higher proliferative activity (81% $Ki67^+$, 40% $BrdU^+$) was demonstrated in pre-B cells. In contrast, sIg^+ B cells were generally $Ki67^-$ and $BrdU^-$.

Phenotypic expression and proliferative activity of thymocytes

In the murine thymus, early observations by Weissman followed the transition from cortical to medullary thymocytes by using administration of ^3H-thymidine in vivo[27]. These observations were recently expanded by Penit who investigated the phenotype of dividing cells after in vivo administration of BrdU[28]. This study utilised a technique to perform double immunofluorescence staining with Abs to BrdU in a biotin-avidin system. $BrdU^+$ blasts were initially located in the cortex and expressed the phenotype $Lyt2^-$, $L3T4^-$ or $Lyt2^+$, $L3T4^+$. A transition from double negative to double positive cells was observed 5 days after BrdU administration. At this time labelled cells were exclusively located in the thymic medulla.

Distinct stages of differentiation are distinguishable in the thymic population in man. Most cortical thymocytes are TdT^+ and express a heterogeneous membrane phenotype. Antigens detected by McAbs of the CD1, CD2 and CD5 groups are found on the surface of these cells. Other functionally relevant structures such as CD4 and CD8 are also present on the same cells and the segregation into $CD4^+$ and $CD8^+$ cells occurs at a later stage of differentiation[29].

A small (3-8 %) subpopulation of cortical thymocytes can be identified by their size and membrane features: these are large cells, with a particularly strong

expression of nuclear TdT and prominent nucleoli and deep blue cytoplasm when stained with May Grunwald-Giemsa[30]. These large cortical thymocytes are strongly $CD7^+$, express CD5 and CD2 heterogeneously and also express HLFA antigens but no CD45 (T200) antigens[21,30,31].

The insertion into the membrane of structures which recognize antigens and transfer these stimulatory signals is a relatively late event in T cell differentiation. McAbs to the T cell receptor are now available[32]. In addition, McAbs to the closely associated CD3 molecules have been characterized. For many years, the study of the CD3 expression on thymocytes has shown a discrepancy. When used in frozen tissue sections of infant thymus, CD3 McAbs appeared to give a wide staining of both medullary and cortical thymocytes[33]. In contrast, when used in cell suspension, only a variable proportion of cells, mostly TdT^-, was stained. This discrepancy has been recently solved by several independent studies which showed that the expression of cCD3 is an early event in T cell differentiation[33-35]. Virtually all $mCD3^-$ cortical thymocytes are $cCD3^+$ when these cells are simultaneously characterized by membrane and intracellular staining using double colour immunofluorescence. Large cortical thymocytes, strongly TdT^+ and $CD7^+$ show a distinct perinuclear rim when stained with CD3 McAbs on cytospins[34,35].

We have posed the question whether cCD3 expression is also detectable in the TdT^+ cells of the BM. Double colour immunofluorescence studies in normal human BM showed that cCD3 and TdT are not found on the same cells[35]. The phenotype of the prothymocytes in man is still not known although Van Dongen and colleagues have reported the existence of a very small proportion of TdT^+ cells in the BM which express $CD7^+$ and have made the hypothesis that these cells may represent prothymocytes[36]. We could not find $CD3^+/TdT^+$ cells even in those samples which had a minute proportion (<5%) of TdT^+ cells which were weakly $CD7^+$.

Previous studies on the proliferative activity of human thymocytes have revealed a high activity within a population of large, $sCD3^-$, cortical thymocytes. We have now applied double and triple colour methods to characterize the BrdU incorporation and Ki67 expression of TdT^+, $CD1^-$ large cortical thymocytes, TdT^+, $CD1^+$ cortical thymocytes and TdT^-, $mCD3^+$ medullary cells, These studies showed that TdT^+, $CD1^-$, $sCD3^-$, $cCD3^+$ and strongly $sCD7^+$ cells represent the most actively proliferating thymocyte subset. More than 90 % of these cells are $Ki67^+$ and >70% are $BrdU^+$. The majority of TdT^+ cortical thymocytes show a lower proliferating activity, 70 % being $Ki67^+$ and 22 % $BrdU^+$. Finally, the majority of TdT^-, $sCD3^+$ cells were resting cells (Ki67 and BrdU <5%)[16,37].

The proliferative activity of malignant lymphoid cell

Similar methods were used to analyse the proliferative activity of leukaemic cells. It was noted that, as in normal BM and thymus, nuclear TdT is present during all the phases of the cell cycle, including the S phase. Secondly, the phenotype of proliferating leukaemic blasts has been characterized. CD19 and CD10 reagents together reacted with >99 % of proliferating blasts in all the null-, common- and pre B cases analysed. In T-ALL, virtually all dividing blasts regularly expressed surface CD7. Therefore in common and null ALL CD19 and CD10 reagents, and in T-ALL CD7 reagents appear to be the optimal for identifying and eliminating dividing leukaemic cells. Finally, the proportion of dividing cells in leukaemic lymphoblasts is generally smaller than it is observed in their normal counterparts (see above and ref. 16,37). This demonstrates that the principle of low leukaemic cell proliferation established in the '60s when the kinetics pattern of leukaemic versus normal myeloblasts were studied with ^{3}H-thymidine[38,39] also holds true for common and T-ALL.

The role of the microenvironment in controlling the differentiation process in the thymus and in the BM is still poorly understood. High proliferative activity and the simultaneous presence of nuclear TdT would suggest that TdT^{+} cells in the BM and cortical thymocytes are at stages of differentiation in which the generation of diversity may take place. It is unlikely that the proliferation of lymphoid progenitors is directly linked to antigen stimulation since their microenvironment lack structures which can present antigen and the immature cells lack membrane receptors for antigen. The factors which control the proliferating activity of these cells are still unknown.

It has been shown that the rearrangement process of the genes coding for Ig and T cell antigen receptor has a high rate of failure[40]. The fate of the failed B and T cells is also unknown although kinetics studies are in agreement with the thought that these cells are destined to die. The high proliferative activity together with the high content of nuclear TdT make immature B cells likely to be susceptible to spontaneous mutations which, together with a high rate of unsuccessful Ig gene rearrangement and the consequent maturation arrest could facilitate the leukaemogenic process[41]. The application of methods to characterize the proliferative status of single cells in order to study the activity of recombinant factors and stromal cells on lymphoid progenitors may provide important pieces of information to further understand leukaemogenesis.

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MONOCLONAL ANTIBODIES FOR DIAGNOSIS AND THERAPY OF LYMPHOPROLIFERATIVE DISEASES*

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Prevention of GvHD in allogeneic bone marrow transplantation

A number of diseases have become curable with bone marrow transplantation (BMT). In allogeneic BMT, in spite of HLA matching, T lymphocytes of the donor may recognize the host's tissues as foreign and initiate graft-versus-host disease (GvHD). GvHD occurs in approximately 50 % of HLA-matched allogeneic BMT and contributes to the fatal outcome in approximately 25 % of cases[1,2].

During the last few years, lymphocyte depletion *in vitro* has been established as a valuable alternative to the use of immunosuppressive drugs in the prevention of GvHD. Monoclonal antibodies (McAbs) provide powerful tools for specifically removing unwanted cells from the suspension of BM cells.

a) Methods for T cell depletion

Well documented studies in animals have shown that GvHD is caused by T lymphocytes[3,4]. A great number of different "cocktails" of McAbs have been used to remove these cells. However, what is referred to as "T cell depletion" in the literature, is often achieved with reagents which also react with non-T cells. For instance, CD2 reagents identify a subset of NK cells[5] and Campath-1 reacts with a wide range of cells including NK cells, B lymphocytes and monocytes[6]. Few purging methods appear to be both reliable and simple for routine use in clinical work. McAbs and rabbit complement (C)[7,8] is one of these methods. Rabbit serum is now available from several commercial sources. The serum is usually tested for the lytic capacity in the presence of C' fixing McAbs, and it is also tested for the intrinsic toxicity to myeloid and erythroid progenitors identifiable *in vitro*. Thirty-day rabbit complement is preferred because at this age the animals have minimal xenospecific cytotoxicity. An attractive alternative to rabbit C' is the use of McAbs with human C' fixing capacity. In this case the source of C' is the patient's own serum and has the advantage of avoiding the standardization of rabbit serum. Some rat McAbs have a powerful human C' fixing capacity[6]. Mouse McAbs of IgM class also work with human C', although often perform more efficiently with rabbit C'[8]. The use of magnetic beads directly conjugated to McAbs or to second layer antisera[9,10] and immunotoxins[11,12] represent valuable alternatives to the C' methods.

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b) Clinical applications

The observations of the clinical applications of McAbs for GvHD prevention are as follows: First, it has been shown that in man, like in animals, GvHD in HLA-matched allogeneic BMT is caused by T lymphocytes alone[7]. Second, the "absolute" elimination of T cells is not necessary and several groups have shown that a depletion leaving $<10^6$/kg T cells to be injected in the patient is sufficient to prevent severe GvHD in HLA-matched patients[7,13]. The elimination of T lymphocytes with the T cell associated McAbs MBG6 or RFT12 (CD6) and RFT8 (CD8) and rabbit C' has effectively prevented severe GvHD in HLA-matched allogeneic BMT[7]. It is not necessary to remove non-T cells, nor to give immunosuppressive drugs post-transplant. In these patients, the adoptive transfer of humoral B cell immunity to tetanus toxoid and hepatitis B has been demonstrated[14].

Two major disadvantages have however been attributed to lymphocyte removal: increase in leukaemia relapse[15,16] and the rejection of grafts. The reported increase in leukaemia relapse after lymphocyte depletion has been observed in studies where T cells (and also non-T cells such as NK cells) were removed and additional immunosuppressive therapy was given post transplant. Thus, the effect of *specific* T cell depletion compared to that of immunosuppressive drugs (e.g. cyclosporin A) has not yet been evaluated critically.

The second drawback attributed to T cell depletion is an increased incidence of failure to engraft or rejection following engraftment[17]. This phenomenon, however, seems to be controllable by adjustments in the conditioning regimen. A recent report illustrates this point. 7 x 2 Gy fractionated irradiation plus cyclophosphamide have yielded take in 26 out of 27 patients receiving HLA-matched T cell depleted marrow[18]. Other regimens such as 7.5 Gy fast rate single dose and cyclophosphamide also seem to ensure good takes[8]. However, when lower total body irradiation (TBI) doses have been delivered (6.9 - 7.2 Gy single dose or 6×2 Gy fractionated), lack of engraftment and rejection has become a problem in an unacceptably high number of transplant patients[18]. Thus it appears that safe 'takes' and prevention of GvHD are achievable in HLA-matched allogeneic BMT although the therapeutic window remains very narrow. A further increase in TBI is associated with considerable toxicity.

This is of particular relevance in mismatched-haploidentical BMT: here GvHD is more difficult to fully prevent and lack of engraftment is more frequent. Thus additional measures are required, without increasing drug-related toxicity. Alternatives such as temporary anaesthesia of the recipient's immune system with HLFA McAbs have produced encouraging results in immunodeficient patients[19]. However, this approach does not appear to be sufficient for

decreasing the complications of haploidentical BMT in patients with leukaemia or aplastic anaemia. Perhaps additional anti-T cell McAbs could help in this field.

A final observation on the effects of specific T cell depletion comes from those studies in which the regeneration of the immune system has been investigated. A slow recovery of T cells has been observed during the 60-150 days post transplant[13]. In previous studies, when T cell depletion was not performed, the absolute number of CD8 cells in peripheral blood rapidly reached abnormally high levels. This resulted in particularly low T4/T8 ratios[20]. The differences of CD8 reconstitution between T cell depletion and other studies may be related to the different regeneration patterns of T cell precursors as opposed to that of inoculated mature cells[13].

Removal of residual malignant cells in autologous BMT

Autologous BMT is associated with the risk of reinjecting neoplastic cells with the patient's own marrow. The number of neoplastic cells present in a BM in "complete remission" is unknown. The concept of "complete remission" is poorly defined, when one considers that different criteria (immunological, cytogenetic and molecular) may be used for identifying residual disease but none of these parameters has been used systematically in assessing the lack of residual leukaemia in the re-injected BM. In most cases of autologous BMT, there is no morphological evidence of BM involvement, and the need of using additional purging methods other than conventional chemotherapy, is based on theoretical considerations rather than facts.

A proportion (1-2%) of the total BM pool is sufficient to reconstitute haemopoiesis after autologous BMT. By using such a small volume of BM harvested in "remission", a dilution effect of malignant cells takes place. Thus, autologous BMT, even without purging, as a rescue after intensive chemoradiotherapy is a rational form of curative anti-neoplastic therapy.

a) The phenotype of malignant lymphoid cells

Phenotypic studies have an important practical application in the diagnosis and therapy of leukaemia and lymphoma. The extensive study of the reactivity of different McAbs on leukaemia and lymphoma blasts has produced simple diagnostic panels[8,21]. The studies on both normal and neoplastic lymphoid cells support the view that particular attention should be paid to the methodology employed. It is well known that in common acute lymphoblastic leukaemia (cALL) and in T-ALL blasts reflect the phenotypes of normal lymphoid precursors in the BM and the thymus, respectively (reviewed in ref. 22). Thus, with methods which permeabilize the cell membranes, cytoplasmic markers such as CD22 for null-ALL and cALL and CD3 for T-ALL should be preferred. In contrast, early surface

antigens such as CD19 and CD7 are optimal when intact cells are labelled in cell suspension. We have compared the cytoplasmic versus surface expression of CD22 (RFB4) and CD3 (CD3a: UCHT1; CD3c OKT3) on 40 cases of null and common ALL. 29 cases of $sCD3^-$ T-ALL and 17 cases of acute myeloid leukaemia (AML) including TdT^+ and/or $CD7^+$ cases[23]. The results of this study are as follows: Firstly, all cases of AML were negative for both cCD22 and cCD3 confirming the lymphoid association of these molecules. Secondly, 2/40 cases of null and common ALL were surface $CD22^+$ and 36 of the remaining 38 cases were $cCD22^+$. T-ALL blasts on the other hand did not express cCD22. Thus, cCD22 is a reliable marker for early cells of the B lineage confirming independent studies[24]. Finally, 29/29 cases of $sCD3^-$ T-ALL were $cCD3^+$. In one case, only UCHT1 (CD3a) was positive while OKT3 (CD3c) was negative. The same blasts also showed an unusual surface phenotype because they strongly expressed Class II. Thus this case may have represented the earliest form of T-ALL in our series. Blasts in null-ALL and cALL were invariably $cCD3^-$, confirming that these antigens are only present in T-lineage cells.

Certain types of leukaemias, such as T-ALL and terminal deoxynucleotidyl transferase $(TdT)^+$ AML express a phenotype which is not found in normal BM. Combinations of McAbs can therefore be used to identify these cells and monitor the remission status of the patient. However, this detailed analysis cannot be performed in many leukaemias and lymphomas since the blasts show phenotypic features which are also found on normal cells in the BM. Methods such as cytogenetics and gene rearrangement studies lack sufficient sensitivity. For these reasons, an answer to the question of the utility of purging will only come from comparative clinical trials.

b) Methods of assessing cytoreduction in leukaemia and lymphoma

Early studies using cell lines of cALL origin and B cell lymphoma origin show that it is possible to remove blasts from normal BM cells with appropriate McAbs and C'[25,26]. However, leukaemic cell lines are suitable only for testing reagents in pre-clinical studies and differ from leukaemic cells in many aspects.

A few "clonogenic assays" performed with fresh leukaemic cells have however also been reported[27,28]. A common feature of these tests is the detection of cells which rapidly divide during early phases of culture. These tests are often cumbersome and difficult to reproduce, and also depend upon the addition of selected batches of factors and animal sera. It is therefore relevant to standardize reproducible and sensitive methods which allow the assessment of the efficacy of blast removal using fresh leukaemia and lymphoma cells, and also to test whether such removal also eliminates the proliferative fraction of these populations.

We have described a technique which is based on the use of independent markers such as TdT for ALL, and Ig or cD22 for B cell lymphoma. The detection of residual target cells is performed with immunofluorescence methods. Malignant cells are mixed with "inert" cells (e.g. the patient's own red blood cells) in approximately equal numbers. The efficacy of lysis can then be assessed by comparing the number of TdT^+ (or Ig^+, $cCD22^+$) cells in the sample after C' lysis with their number in a control (irrelevant Ab) sample for a given number of "inert" cells[8].

Double colour immunofluorescence on cytospins has been shown to be a sensitive method for precisely identifying residual blasts. Dilution experiments have previously demonstrated that 1 in 10^4 residual TdT cells could be identified with these methods[29]. We have confirmed these studies with dilution experiments. Double colour IF was compared to single colour cell sorter and microscope analysis, as well as to molecular studies for Ig-gene rearrangements studies in order to assess the efficacy of detecting common ALL blasts admixed with normal peripheral blood. The double colour immunofluorescence technique was shown to be the most sensitive method for detecting residual cALL blasts[23].

c) The selection of optimal reagents

The classification of reagents by the Leucocyte Differentiation Antigens Workshops[30,31,32] have facilitated the task of scientists who wish to use McAbs to eliminate unwanted cells. McAbs recognizing the same epitopes but of different class and subclass can be chosen according to the individual aims.

When a C' lysis system is used, it is important to determine the C' fixing capacity of each individual reagent. We have tested the reagents sent to the Third Leucocyte Differentiation Antigens Workshop and classified within the CD10, CD19 and CD20 clusters for their reactivity and ability to fix human and/or rabbit C'. The conclusions of this study are as follows: Firstly, CD10 McAbs reacted with TdT^+ cells and CD19 Abs reacted with TdT^+, pre-B and $sIgM^+$ cells in the BM. Antibodies of the CD20 cluster and unclustered reagents such as RFB7 and Y29/55 were B cell specific but appeared on the cell surface at a slightly later stage of B cell differentiation. Secondly, these Abs reacted with fresh leukaemia and lymphoma cells representing the corresponding staging of B cell differentiation. Finally, the reagents which performed better in terms of rabbit C' fixation were RFAL3 (CD10), SB4 (CD19), B-C1 (CD20), RFB7 and Y29/55 (unclustered; ref. 33). RFAL3, B-C1 and RFB7 are also human C' fixing while SB4 has little human C' fixing capacity on its own but can improve that of other reagents when used in combination[34]. The conclusion of these studies is that in common ALL and null ALL a combination of RFAL3 and SB4 is optimal, while for the elimination of lymphoma cells a combination of RFB7 and SB4 with the possible addition of

other reagents such as B-C1 or Y29/55 can be recommended. Independent studies have also shown the suitability of the CD19-CD20 cocktail for B cell lymphoma[35]. Most of these detailed investigations have concentrated on the comparison of C' fixing McAbs.

d) Investigation of individual patients

The antigenic expression of malignant cells is heterogeneous. Cocktails of Abs are used in order to overcome this heterogeneity. We have investigated the elimination of leukaemia and lymphoma cells with the different combinations of the McAbs selected and human and/or rabbit C'. These investigations are performed on fresh leukaemia and lymphoma samples. When lymph nodes of lymphoma patients are studied, a cell suspension is obtained either by treating the nodes mechanically or enzimatically. With these methods we have now studied >100 cases of ALL and B cell lymphoma at presentation or in relapse. The selected McAbs are very efficient in eliminating malignant cells: e.g. with a combination of RFAL3+SB4 and rabbit C' >4 log lysis has been achieved in >70% of null and cALL cases. These Abs also eliminated the proliferative fraction of leukaemic cells (Janossy G, Campana D et al, manuscript in preparation).

e) Clinical observations

Many factors such as the use of more aggressive conditioning regimes and the improvement of cryopreservation and transfusion technology contribute to the success of an autologous BMT. The toxicity of this procedure is now comparable to that of conventional chemotherapy. As a consequence, a number of patients may benefit from an expanding autologous BMT programme.

A series of pilot studies have provided the necessary preliminary information on the effects of purging in autologous BMT. Early clinical studies of purging in ALL and B cell lymphoma using McAbs and rabbit C' [33,36-38] indicated the feasibility and the safety of the procedure. Recently published results in patients with non-Hodgkin's lymphoma with poor prognosis treated with autologous BMT that had been purged with B1 McAb confirm the safety of the procedure in older patients. The median age in this study was 42 yrs[39].

A clinical collaboration has been established between our Department and the groups at the University Hospital, Uppsala and at the Royal Infirmary and Hospital for Sick Children, Glasgow. In this trial the selection of patients is based on the following criteria: (a) ALL patients are transplanted in first remission when at high risk of relapse (e.g. $>100 \times 10^9$ WBC at presentation) or in second or subsequent full remissions. (b) The phenotype of blasts cells is characterized at presentation or in relapse. This is performed with the same reagents which are used for purging, and the sensitivity of the blasts to C' lysis is also assessed using the methods described. The optimal combination of reagents which are found to be

capable of achieving >4 log cytoreduction is used for purging. (c) Additional pieces of information are the detection of residual target cells after purging with sensitive methods and, (d) the assessment of the CFU-GM injected into the patient. Into this pilot trial 19 patients (11 children and 8 adults) have been entered in first (4 patients) and second (14 patients) or subsequent (1 patient) remissions. The follow-up is >30 months. Sixteen out of 19 patients are alive and well. Thirteen of these are now beyond 12 months follow-up. In 4 other patients conditioning prior autologous BMT was carried out in relapse. All 4 patients in this second group relapsed within the first year after BMT.

A number of conclusions can be drawn from this study. Firstly, post transplant complications were minimal confirming the low toxicity of the procedure. Secondly, the purging procedure effectively eliminated target cells even in patients with detectable BM relapse but still allowed a hemopoietic recovery. This recovery was as fast as seen in patients who received unpurged BM[40]. Finally, no early relapses have been observed in the patients transplanted in remission. The 3 relapses occurred after 9, 10 and 12 months of transplant. We tentatively conclude that many early relapses seen in other studies might be due to considerable residual disease which may be detectable at the time of transplant with the immunological techniques used in our study.

At this stage, it will be necessary to organize large collaborative trials in order to directly compare autologous BMT protocols with or without purging. To obtain a credible answer from these studies, the purging methods employed must be of proven efficacy and safety.

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