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70 Bukowska Street, room 104
60-812 Poznan, Poland
phone/fax: +48 61 854 72 74
email: nowinylekarskie@ump.edu.pl
www.jms.ump.edu.pl

DISTRIBUTION AND SUBSCRIPTIONS
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60-356 Poznan, Poland
phone/fax: +48 61 854 64 87
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60-812 Poznań, ul. Bukowska 70
tel./fax: +48 61 854 71 51

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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

Ethical guidelines

The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

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Complete Balanced Atrioventricular Septal Defect – Results of Bi-ventricular Surgical Correction

Michał Sobieraj[1], Michał Szemień[1], Marcin Bednarek[1], Michał Wojtalik[2], Wojciech Mrówczyński[2]

[1] Pediatric Cardiac Surgery Students' Interest Group, Poznan University of Medical Sciences, Poland
[2] Department of Pediatric Cardiac Surgery, Poznan University of Medical Sciences, Poland

ABSTRACT

Aim. The aim of this study was to analyze the results of surgical treatment of balanced AVSD during 10-year period in a single center. The adequacy of atrioventricular valves reconstruction was scrutinized as well as risk factors for mortality, redo surgery, pulmonary hypertension incidence were assessed.
Materials and method. One hundred twenty one patients underwent surgical correction of AVSD between 2004 and 2013. All patients were operated by double patch technique with the apposition zone closure. Patients' median age was: 109 (86–151) days, body weight: 4.5 (4–5.5) kg. Down syndrome was present in 100 (82.6%) patients.
Results. Early mortality equaled 17.3% (21 patients) and late mortality was 1.7% (2 patients). Pulmonary hypertension (42.9%) and heart failure (33.3%) were the main causes. Cardiopulmonary bypass time ($p < 0.0001$), aortic cross-clamping time ($p = 0.003$), inotropic index value ($p < 0.0001$), maximal mean pulmonary artery pressure ($p = 0.008$) were significantly higher in the group of patients who died. Ten patients (8.3%) required early reoperation and nine (9%) required late reoperation. Early postoperative mitral valve regurgitation of III-rd or higher degree was risk factor for early and late reoperations ($p = 0.0002$). The probability of freedom from all reoperations at 6 months, 1 and 5 years was respectively: 0.83; 0.80 and 0.71.
Conclusions. Pulmonary hypertension and low cardiac output syndrome have significant influence on results of AVSD treatment. The predisposing risk factors are prolonged time of surgical procedure and presence of pneumonia. Mitral valve reconstruction quality has an influence for early and late results.

Keywords: AVSD, mitral valve regurgitation, redo surgery.

Introduction

Atrioventricular septal defect (AVSD) is a complex cardiac malformation comprising entire spectrum of different defects: isolated cleft of anterior mitral valve leaflet, ostium primum atrial septal defect, ostium primum atrial septal defect with ventricular septal defect (VSD), transitional AVSD type, and finally a complete AVSD. The later can occur in more severe unbalanced form.

Anatomy and pathophysiology of the malformation causes 60–65% mortality rate in first year of life and over 90% in first two years. Thus, the only definitive treatment is a surgical correction with reconstruction of atrioventricular valves – the most crucial part of surgical approach. Even though progress in cardiac surgery techniques improved outcomes of patients with AVSD including quality of life, still 15–20% patients require reoperation [1–3]. The most common indication for reoperation after initial repair is a regurgitation of atrioventricular valves, particularly mitral insufficiency which is the cause of reoperation in 70–90% [2, 4, 5].

Surgical correction of AVSD can be complicated by other different problems including: post-operative pulmonary hypertension (PH), low cardiac output syndrome (LCOS) and III° atrioventricular heart block.

Aim

The aim of the study was to assess the results of surgical bi-ventricular correction of complete AVSD during 10-year period in a single unit. The risk factors of atrioventricular valves regurgitation, intraoperative mortality, pulmonary hypertension and other complications were studied.

Materials and method

Patients

One hundred twenty one patients underwent surgical correction of AVSD in the Department of Pediatric Cardiac Surgery in the University of Medical Sciences, Poznań, between 2004–2013. Patients with isolated cleft of anterior mitral valve leaflet, partial, transitional and unbalanced forms of AVSD were excluded. Medical records (surgical reports, case histories, local medical data base and local data from National Cardiac Surgery Register) were reviewed.

Ten patients underwent pulmonary artery banding before primary correction. Seven patients (5.79%) had coexisting malformations like pulmonary valve stenosis (n = 3; 2.5%), tetralogy of Fallot (n = 1; 0.8%), and coarctation of the aorta (n = 1; 0.8%). Demographic data are shown in **Table 1**.

This observational study has been approved by the local Ethics Committee (resolution: 32/13 from 03.01.2013) and an individual patients' consent was waived.

Table 1. AVSD patients characteristics

Demographic variables	
N	121
Gender (female/male)	58 (47.9%) / 63 (52.1%)
Down's syndrome	100 (82.6%)
Age (days)	109 (86–151)
Body weight (kg)	4.5 (4–5.5)
Body surface area (m^2)	0.26 (0.24–0.30)
Intraoperative variables	
CBP (min)	119 (103–138)
Ao-x-clamp (min)	74 (63–88)
Postoperative variables	
ICU time (days)	10 (6–20)
Mechanical ventilation (hours)	96 (32–266)
INO SCORE (mcg/kg/min)	15 (6.70–24.85)
Max. mPAP (mmHg)	49.2 ± 18.2

CBP – cardiopulmonary bypass time; Ao-x-clamp – aortic cross-clamp time; INO SCORE – inotropic index; ICU time – intensive care unit stay time; Max. mPAP – maximal mean pulmonary artery pressure

Surgical technique

All patients were operated through the median sternotomy by applying two-patch technique [6, 7], with the use of aorto-bicaval cannulation for cardiopulmonary bypass in moderate hypothermia. Cardiopulmonary bypass time (CBP) and aortic cross clamping time (Ao-x-clamp) were recorded. Ventricular septal defect was closed with Gore-Tex patch attached to interventricular septum by a continuous suture. Then the division and reconstruction of atrioventricular valves was performed. The left-sided apposition zone of bridging leaflets was sutured with interrupted 6–0 polypropylene monofilament sutures. Autologous pericardium was applied for atrial septal defect closure. Regurgitation of reconstructed atrioventricular valves was tested saline injection into the ventricle and by the use of intraoperative transesophageal echocardiography.

Post-operative care

All patients received standard intensive care unit treatment. When necessary pulmonary artery pressure was recorded by a catheter inserted through the infundibulum of the right ventricle. Pulmonary hypertension was treated by hyperventilation, nitric oxide inhalation, and phosphodiesterase V inhibitors administration via nasogastric tube (sildenafil). Pharmacological circulatory support was obtained by catecholamines and vasodilatators: adrenaline, isoprenaline, dopamine, dobutamine and milrinone. The degree of catecholamine support was expressed by inotropic index [8, 9], defined as: INO-SCORE (mcg/kg/min) = DPA+DBX+100xISO+100xADR, (where: DPA = dopamine, DBX = dobutamine, ISO = isoprenaline, ADR = adrenaline). One patient required mechanical circulatory support – ECMO due to heart failure refractory to conventional treatment.

Echocardiographic study

Transthoracic echocardiography was performed in all patients preoperatively, pos-operatively and during the follow-up. Left ventricular ejection fraction (LVEF), left ventricular shortening fraction (SF), common atrioventricular valve regurgitation (CAVVI), mitral (MI) and tricuspid (TI) valve regurgitations were analyzed.

Follow-up

Transthoracic echocardiography and medical examination were performed in all patients during the follow-up. Median observation time was 714 days (420–1002). Total follow-up was 143.5 patient-years.

Statistical analysis

Normality of data distribution was tested by Kolmogorov-Smirnov test. Continuous variables were presented as mean and standard deviation or median and range according to the distribution normality. T-student and Mann-Whitney tests were used for intergroup independent comparisons. Fisher's exact test was employed for categorical variables comparisons. Kaplan-Meier method was applied to assess the probability of freedom from all reoperations and reoperations for significant mitral valve regurgitation, 95% confidence interval was calculated. *P*-value less than 0.05 was considered statistically significant.

Results

Mortality

Thirty-day mortality equaled 17.3% (21 patients). Nine patients died from pulmonary hypertension (42.9%), 7 from heart failure (33.3%), 2 from coexisting pulmonary hypertension and heart failure (9.5%). Multiple organ dysfunction syndrome was the cause of 3 deaths (14.3%). Late mortality (> 30 days) was 1.7% (2 deaths). Both patients died from coexisting pulmonary hypertension and heart failure.

Prolonged cardiopulmonary bypass time, prolonged aortic cross-clamping time, higher inotropic index and higher maximal mean pulmonary artery pressure were influencing negatively the survival with statistical significance (**Table 2**). Demographic data had no statistically significant impact on the survival. There were no statistically significant differences in: pre-operative left ventricle ejection fraction, left ventricle fractional shortening, mechanical ventilation time and presence of common atrioventricular valve regurgitation (III-rd degree or higher) in patients who died and patients who survived. There were no early and late deaths in 10 patients who underwent pulmonary artery banding before total correction in comparison to children without banding (early mortality 0% vs. 18.9%) but this difference was not significant (p = 0.207).

Reoperations

Ten patients required early reoperation (⩽30 days). Nine (9%) patients out of 100 who survived early postoperative time required late reoperation (>30 days). Four patients required second redo surgery. One patient underwent third reoperation. Causes of reoperations and re-interventions are listed in **Table 3**. The usual redo strategy for mitral valve regurgitation was repeated mitral valve reconstruction.

The median time of early reoperation equaled 14 days (7–24). The median time of late redo surgery was 205 days (52–950). The probability of freedom from all reoperations at 6 months, 1 and 5 years was respectively: 0.83 (0.75–0.92; 95%CI); 0.80 (0.70–0.90; 95%CI) and 0.71 (0.56–0.90; 95%CI) (**Figure 1**). **Figure 2** shows freedom from the first reoperation due to the significant mitral valve regurgitation with the probability of 0.87 (0.80–0.96; 95%CI); 0.85 (0.77–0.95;

Table 2. Mortality risk factors in patients undergoing AVSD correction

Variables	Survival	Death	*p*-value
Intraoperative variables			
CBP (min)	113 (101–130)	144 (129–179)	< 0.0001
Ao-x-clamp (min)	71 (62–85)	91 (76–103)	0.0003
Postoperative variables			
INO SCORE (mcg/kg/min)	12.9 (6.0–19.1)	58.5 (19.3–87.5)	< 0.0001
Max. mPAP (mmHg)	44.8 ± 17.4	61.0 ± 16.4	0.008
Mechanical ventilation (hours)	84 (32–244)	174 (19–573)	0.203
Demographic variables			
Age (days)	108 (87–153)	113 (69–172)	0.424
Body weight (kg)	4.55 (4–5.9)	4.15 (3.8–5)	0.207
Body surface area (m^2)	0.27 (0.24–0.31)	0.26 (0.24–0.28)	0.132
Down's syndrome	84.7%	69.6%	0.129
Preoperative echocardiographic data			
CAVVI ⩾ III-rd degree	32.3%	45.4%	0.320
LVEF (%)	78 (71–84)	78 (73–81)	0.812
SF (%)	43 (38–50)	43 (38–46.8)	0.951

CBP – cardiopulmonary bypass time; Ao-x-clamp – aortic cross-clamp time; INO SCORE – inotropic index; Max. mPAP – maximal mean pulmonary artery pressure; CAVVI – common atrioventricular valve regurgitation; LVEF – left ventricular ejection fraction; SF – left ventricular shortening fraction

Table 3. Reoperations in patients undergoing AVSD correction

Early reoperations	
MI	4
MI+TI	1
VSD	1
Pacemaker implantation	4
Late reoperations and interventions	
MI	6
MI+TI	2
Wound revision	1
Pleural effusion drainage	1
Pericardial effusion drainage	1
Pacemaker implantation	3

MI – mitral valve regurgitation; TI – tricuspid valve regurgitation; VSD – ventricular septal defect

95%CI) and 0.77 (0.61–0.97; 95%CI) at 6 months, 1 and 5 years respectively.

Patients with early (⩽ 30 days) III-rd or higher degree mitral valve regurgitation required significantly more reoperations than other children (22% vs. 0%) (p = 0.0002). Patients' age, body weight, body surface, cardiopulmonary bypass time, aortic cross-clamping time, inotropic index, maximal mean pulmonary artery

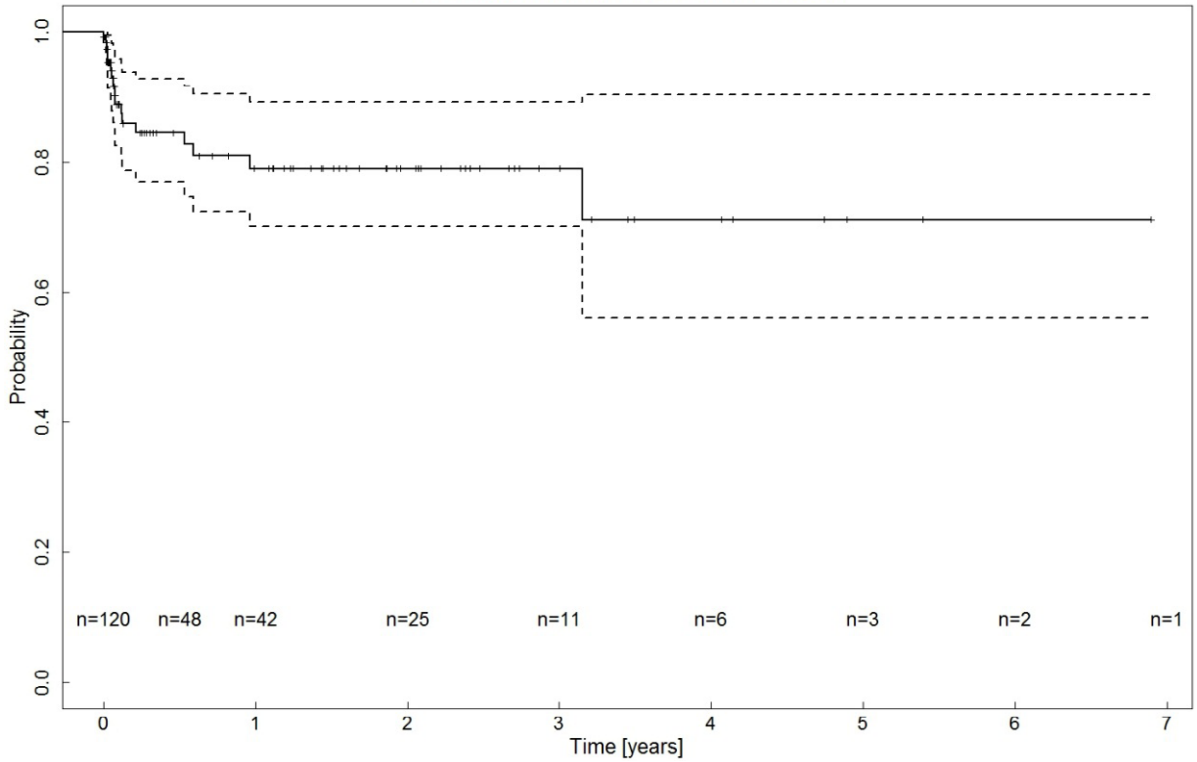

Figure 1. Freedom from the first reintervention

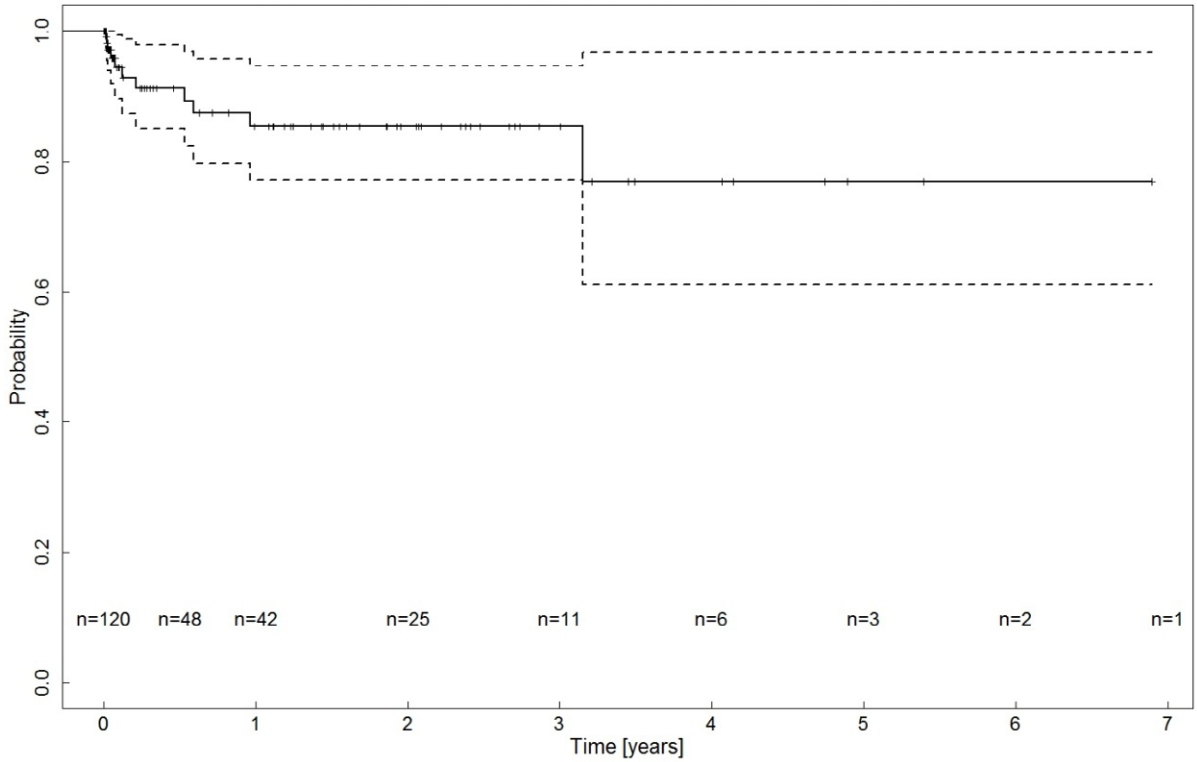

Figure 2. Freedom from the first reintervention due to mitral regurgitation

pressure, left ventricle ejection fraction, left ventricle fractional shortening were not significantly different in the groups of children requiring redo mitral valve surgery and in the group of patients without reoperation. Presence of preoperative III-rd or higher degree common atrioventricular valve regurgitation was not significantly more frequent in patients undergoing redo surgery (**Table 4**).

Patients who underwent pulmonary banding as a first stage of treatment did not require early neither late reoperations in comparison to the rest of the cohort (0% vs. 17.12%). However, this comparison was not statistically significant (p = 0.360).

Atrioventricular valve function

Ninety five (78.5%) patients had pre-operative common atrioventricular valve regurgitation, forty patients (34.8%) had III-rd or higher degree incompetence. In the first post-operative echocardiographic study 45 patients (44.5%) had III-rd or higher degree mitral valve regurgitation, whereas IV-th degree mitral valve regurgitation was present in 12 patients (12%) – 5 of them required early reoperation. During the follow-up 8 patients who had significant mitral valve regurgitation required reoperation. In last control echocardiographic study 5 patients had III-rd degree mitral valve regurgitation but did not require reoperation at the end of observation. The IV-th degree mitral valve regurgitation was not noted at the follow-up conclusion.

Post-operative complications

Fifty two patients (43%) had pulmonary hypertension in the immediate post-operative period. Thirty three patients (27.3%) required nitric oxide inhalation or phosphodiesterase inhibitor V treatment concomitant to hyperventilation. Pulmonary artery banding resulted in significantly less frequent incidence of pulmonary hypertension during the total AVSD correction (1% vs. 45.9%; p = 0.042), only one patient who had pulmonary hypertension in this group required nitric oxide treatment.

Twenty one patients (17.4%) had pulmonary hypertensive crisis (PHC). Mortality rate, cardiopulmonary bypass time, mechanical ventilation time, intensive care unit stay time, inotropic index, maximum mean pulmonary artery pressure were significantly higher and incidence of pneumonia more frequent in patients with PHC than patients without it (**Table 5**). In contrary, the differences in demographic variables between these two groups were not statistically relevant.

Low cardiac output syndrome that required pharmacological support was present in 33 patients (27.27%). Cardiopulmonary bypass time, aortic cross-clamping time, mechanical ventilation time and inotropic index were significantly higher in patients with LCOS than patients without it (**Table 6**).

Among other post-operative complications pneumonia (48%), metabolic acidosis (15.7%), respiratory distress syndrome (5%) and sepsis (1.6%) were recognized.

Table 4. Reoperation risk factors in patients undergoing AVSD correction

Variables	Reoperation	No reoperation	*p*-value
Demographic variables			
Age (days)	105 (69–117)	112 (88–174)	0.133
Body weight (kg)	4.35 (3.9–5)	4.5 (4–5.9)	0.293
Body surface area (m^2)	0.26 (0.23–0.28)	0.27 (0.24–0.31)	0.186
Intraoperative variables			
CBP (min)	139 (113–157)	118 (103–136)	0.223
Ao-x-clamp (min)	88 (67–96)	74 (63–88)	0.223
Postoperative variables			
INO SCORE (mcg/kg/min)	22 (6–32.3)	14.3 (6.53–23)	0.173
Max. mPAP (mmHg)	52.31 ± 14.9	43.62 ± 18.74	0.295
Preoperative echocardiographic data			
CAVVI ⩾ III-rd degree	45.4%	34%	0.513
LVEF (%)	76 (67–85)	78 (72–83)	0.866
SF (%)	45 (35–51)	43 (38–48)	0.702

CBP – cardiopulmonary bypass time; Ao-x-clamp – aortic cross-clamp time; INO SCORE – inotropic index; Max. mPAP – maximal mean pulmonary artery pressure; CAVVI – common atrioventricular valve regurgitation; LVEF – left ventricular ejection fraction; SF – left ventricular shortening fraction

Table 5. Pulmonary hypertensive crisis risk factors in patients undergoing AVSD correction

Variables	PHC (100)	No PHC (21)	*p*-value
Intraoperative variables			
CBP (min)	115 (102–133)	130 (121–160)	0.015
Postoperative variables			
Mortality	12%	50%	0.0001
Mechanical ventilation (hours)	72 (27–168)	384 (221–585)	< 0.0001
ICU time (days)	9 (6–16)	24 (11–28)	0.002
INO SCORE (mcg/kg/min)	14.0 (6–20.7)	32.3 (15–98)	0.003
Max. mPAP (mmHg)	40.6 ± 9.6	65.9 ± 15.8	< 0.0001
Pneumonia	41%	81%	0.0013
Demographic variables			
Age (days)	110 (85–147)	106 (84–174)	0.719
Body weight (kg)	4.5 (4–5.9)	4.6 (3.9–5.5)	0.795
Body surface area (m^2)	0.27 (0.24–0.31)	0.26 (0.24–0.29)	0.548
Gender (female/male)	50%/50%	38%/62%	0.348
Down's syndrome	83%	81%	0.760

CBP – cardiopulmonary bypass time; INO SCORE – inotropic index; ICU time – intensive care unit stay; Max. mPAP – maximal mean pulmonary artery pressure

Table 6. Low cardiac output syndrome in patients undergoing AVSD correction.

Variables	LCOS	No LCOS	*p*-value
CBP (min)	113 (101–130)	130 (117–164)	0.0002
Ao-x-clamp (min)	71 (62–86)	81 (68–93)	0.035
Mechanical ventilation (hours)	77.5 (29–217)	168 (72–439)	0.030
INO SCORE (mcg/kg/min)	12.9 (6–18.7)	30.2 (18.8–52.3)	0.0001

LCOS – low cardiac output syndrome; CBP – cardiopulmonary bypass time; Ao-x-clamp – aortic cross-clamp time; INO SCORE – inotropic index

Discussion

This study analyzes the outcomes of complete AVSD correction in 121 patients in a single unit. All patients were operated with the double patch technique, which is commonly applied in many units [3, 5, 10–12]. The advantages of this method are: simpler partitioning and anchorage of bridging leaflets without a need for leaflet incision as well as potentially better protection of cardiac conduction system compared to single-patch and no patch technique. However, shorter cardiopulmonary bypass time and aortic cross-clamping time during single-patch and no-patch technique in comparison to double-patch approach can be an advantage of the formers [13]. The use of single patch/no-patch method is limited when additional intra-cardiac malformations like tetralogy of Fallot or double outlet right ventricle are present [13]. Moreover, the use if these techniques is connected with a frequent incidence of residual VSD and certain difficulties in attaching leaflets to Gore-Tech patch, which may result in dehiscence of sutured tissues, particularly in their mitral part [5, 14, 15].

Thirty-day mortality rate was 17.3%. Although this results are comparable to the rates from other reports [2, 10, 16], it is possible to achieve mortality rates of 0–1.4% as reported by Bakhtiary [12] or Xie O [17]. In this study the main cause of early death was pulmonary hypertension (42.9%). This observation is in contrast to other reports where presence of pulmonary hypertension was not such a pronounced risk factor of early death. For example, Prifti [18] reports of only 12.5% deaths caused by pulmonary hypertension, whereas the study by Gunter [5] shows no early deaths related to this complication. Risk factors analysis shows that presence of pneumonia have significant influence on pulmonary hypertension and the PHC development [19]. Other risk factors predisposing to PHC included prolonged cardiopulmonary bypass time and high inotropic index value.

The most frequent cause of early death in patients undergoing surgical correction of AVSD is a heart failure. It contributes to more than 50% of early mortality [2, 3, 5, 16, 18]. In our study the heart failure was the cause of 33% deaths. Prolonged aortic cross-clamping time and prolonged cardiopulmonary bypass time were risk factors for low cardiac output syndrome. Consistent risk factors for in-hospital mortality were not defined so far. Prolonged cardiopulmonary bypass time, pro-

longed aortic cross-clamp time, pulmonary pressure value and inotropic index value were the risk factors for in-hospital mortality. This observation is corresponding to other reports [11, 18, 20]. However, some studies are opposite to it [2, 16].

The demographic variables were not risk factors for mortality in this study. Although this observation is similar to other studies [2, 3, 16, 18, 21], there are some contrary reports [3, 5]. There were no early or late deaths in patient group who underwent pulmonary artery banding. No statistically significant differences in mortality were recorded between group of patients with PAB and patients without it. It can be explained by small group of patients who underwent pulmonary artery banding before primary correction (10 patients with PAB vs 111 without it). Similar results is reported by Gunter's [5] study.

Ten patients from our cohort (8.3%) required early reoperations and 9 patients (9%) required late reoperations. This reoperations rate is similar to the results reported by others [3, 5, 16–18]. The probability of freedom from re-interventions at 6 months, 1 and 5 years were 0.83; 0.80 and 0.71 respectively and it is similar to other studies [2, 16, 20]. Mitral valve redo surgery was necessary in 13 patients (54.2% of 24 reoperations including second and third reoperation). Early mitral valve regurgitation had influence on early or late reoperations frequency which is comparable to other reports [3, 10, 18]. The demographic and preoperative echocardiography variables were not a risk factors for development of mitral valve regurgitation. However, some studies define patients age, body weight and the absence of Down's syndrome as risk factors for increased reoperation rate [2, 10, 16–18]. Hoohenkerk [3] and Prifti [18] reported pre-operative atrioventricular valve regurgitation as significant risk factors for the reoperation. Some authors indicate that the closure of the apposition zone can decrease the mitral valve reoperation rate [3, 18, 22]. However, this aspect was not scrutinized as the apposition zone was always sutured during each AVSD correction in this study.

Study limitations:

This study has the following limitations. It is a retrospective observational study comprising 10-year period. Cardiopulmonary bypass and cardio-protection techniques (introduction of blood cardioplegia) have evolved during that time. It could have affected the statistical analyses. The next limitation arises from the fact that the procedures were performed by different surgeons which certainly could have had influenced the results. However, taking into account all consecutive interventions allows a global assessment of AVSD treatment results in a single unit.

Conclusions

The early results of AVSD treatment are influenced by complications occurring in the early postoperative period including pulmonary hypertension and low cardiac output syndrome. The predisposing risk factors are prolonged cardiopulmonary bypass time, prolonged aortic cross-clamping time and presence of pneumonia.

Impaired atrioventricular valvular function still remains a significant problem. Mitral valve reconstruction quality has an influence for early and late results. Mitral valve regurgitation in the early post-operative period affects early and late reoperation rate.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Correspondence address:
27/33 Szpitalna Str.
60-572 Poznań, Poland
phone: +48 61 8491277

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Evaluation of radiological parameters after distal radius fracture in elderly people

Adam Zaręba

Department of Orthopedics and Trauma Surgery, General Hospital in Szczecinek, Poland

ABSTRACT

Introduction. Distal radius fractures are one of the most frequent problems in every day practice and almost 17% of all fractures in adults. We have to very often face effects of low-energy trauma in elderly people. Osteoporotic bone and composed morphology of fracture forces surgeon to consider operation among the best options of treatment.

Aim. This is an attempt to compare radiological results achieved by closed reduction and cast immobilization versus open reduction and internal fixation with „Aptus®" (Medartis, Basel Switzerland) volar plate, as well as dynamics of redisplacement during immobilization based on radiological parameters of distal radius.

Material and methods. There were 101 patients in examined group, aged 60 to 91.

Results. Internal fixation lead to smaller secondary displacement comparing to cast immobilization, but significancy varies depending on fracture morphology. Redisplacement during cast immobilization was comparable during first seven days and following weeks previous to removal of splint.

Keywords: distal radius, fracture, elderly, operative treatment.

Introduction

Distal radius fractures are one of the most frequent problems in every day practice and almost 17% of all fractures in adults [1]. We have to very often face effects of low-energy trauma in elderly people [2, 4]. Osteoporotic bone and composed morphology of fracture forces surgeon to consider operation among the best options of treatment.

Risk factors of distal radius fracture are osteoporosis, age, female gender, malnutrition as well as diseases leading to discoordination and causing greater risk of falling down [3]. There have been noticed greater occurrence during winter months [5].

Despite the high morbidity there is still no agreement when to choose more invasive methods in elderly people. Due to diametrically different biological condition of patients, from self sufficient to totally dependent, individual approach is needed. This is an attempt to compare results achieved by closed reduction and cast immobilization versus open reduction and internal fixation with „Aptus®" (Medartis, Basel Switzerland) volar plate, as well as dynamics of redisplacement during immobilization based on radiological parameters of distal radius.

Material and methods

There were 101 patient after distal radius fracture in examined group, aged 60 to 91. 51 were treated operatively and 50 conservatively. Average age was respectively 69.9 and 72.8. In first group there were 49 woman and 2 men operated with "Aptus" volar plate. Indications for surgery were:

- palmar displacement
- dorsal displacement over 20°
- compression on dorsal cortex
- intraarticular fracture
- unsuccessful reposition
- redisplacement revealed in follow-up care.

Second group consisted of injured immobilized in the cast. It was satisfactory reposition, disqualification from surgery due to general condition or patient's per-

sonal preferences that decided about assigning to this part. Patients with co-existing distal ulna fracture other than styloid process were excluded. Each time there was closed reposition performed to avoid soft tissue damage, as well as to decrease edema, pain and contracture. Morphology of fractures was classified accordingly to AO references (**Figure 1, Table 1**).

All patients underwent similar conservative treatment. In A&E department hematoma block was performed by injecting approximately 15 cc of lidocaine into fracture gap. Sedation with midazolam and fentanyl when required. Next reduction was performed using Sokolowski's apparatus, and after applying cast patients went for control X-Ray scan. Follow-up visits were scheduled for 7th and 28th day after fracture. If needed immobilization was prolonged for two additional weeks. Due to poor availability of rehabilitation no proper protocol could be used.

Patients qualified for operation were treated by open reduction and internal fixation with „Aptus" plate by Medartis. Two millimeter volar locking compression plate was used. Implant is made of titanium ASTM F136, what enables patient for MR examination, as well as reduces risk of immunologic reactions, infections and forming biofilm on its surface [6].

Approach was performed radially to flexor carpi radialis tendon, as described by Henry. It gives propper visualization of volar cortex, which is crucial for restoring radial length, and allows firm screws placement in subchondral bone. After fixation plate is covered by pronator quadratus to prevent tendon irritation. Volar approach is first choice in instable fractures with volar displacement of distal fragment or if carpal tunnel decompression is needed. Appropriate implant localization is confirmed with image intensifier in P-A and lateral projections with additional radiocarpal joint scan. No drainage was performed. After pneumatic tourniquet was released few minute pressure was applied on the wound. Transverse carpal ligament was released only in patients with preoperative carpal tunnel syndrome [8, 9]. After operation patients spent one or two days in the ward, when radiological control was performed and rehabilitation instructions given. In first 6 weeks only weight – free active and passive exercises were advised. Sutures were removed after 12 to 14 days. After 6 weeks and control X-ray scan patients were allowed to use hand freely (**Figure 2, Table 2**).

X-ray scan were assessed using calibrated measurement in "Exhibeon" 2.7. Four parameters were estimated – radial height, radial inclination, volar tilt and ulnar variance. In conservatively treated patients there was evaluated difference between the status after reposition and follow-up controls after 7 days and 4 to 6 weeks. In operated patients radiological assessment was conducted after surgery and after 6 weeks. Each parameter was measured twice to avoid measurement errors [10]. Obtained data was analyzed with Graph Pad Prism 5.1. Shapiro – Wilk's test revealed no normal distribution in both groups. Man – Whitney's was used to assess significancy of differences in both groups.

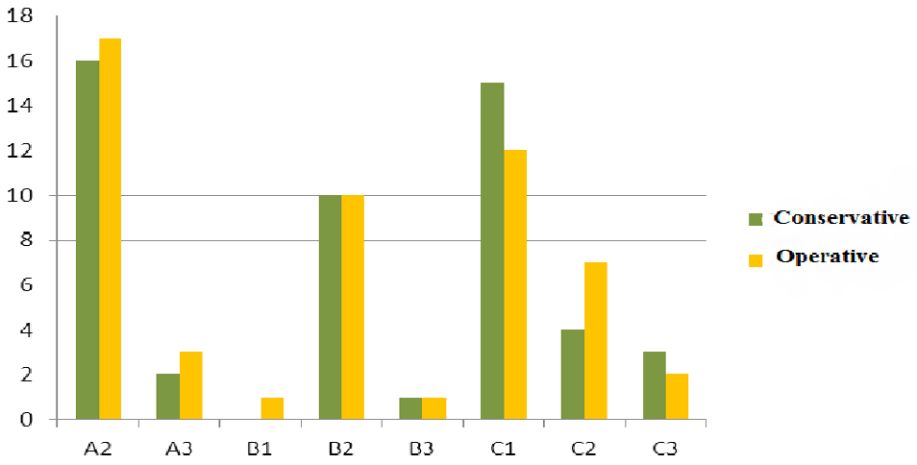

Figure 1. Distribution of fracture cases according to AO classification

Table 1. Distribution of fracture cases according to AO classification

	A2	A3	B1	B2	B3	C1	C2	C3
Conservative	16	2	0	10	1	15	4	3
Operative	17	3	1	10	1	12	7	2

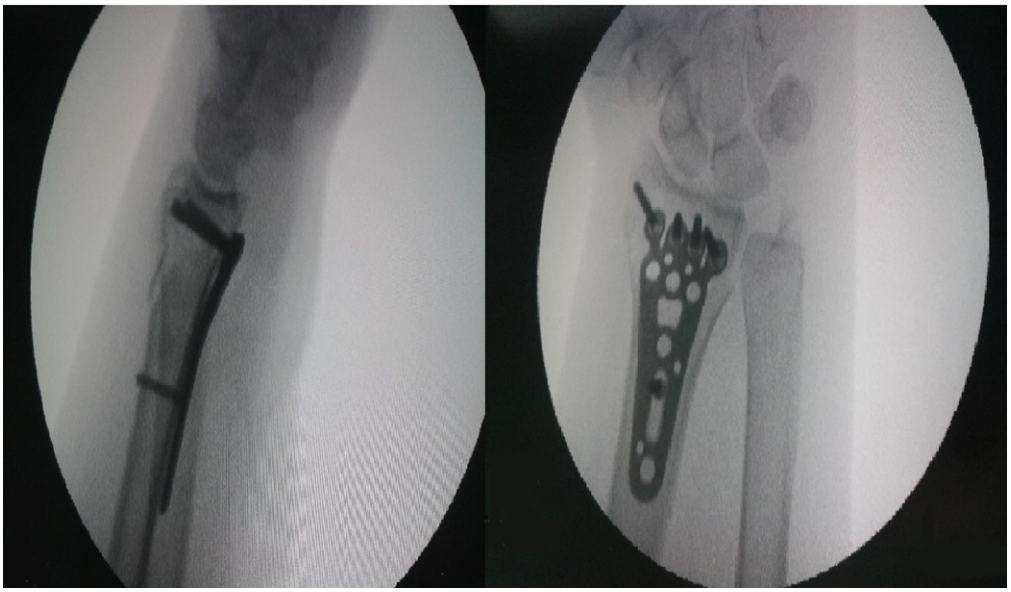

Figure 2. Confirmation of proper placement of the plate

Table 2. Average radiological parameters obtained after reposition in both groups

	Height (mm)	Tilt (deg)	Inclination (mm)	Variance (deg)
Conservative	10.3	1.9	18.6	-1.2
Operative	10.2	6.5	16.9	-0.5

Wilcoxon's test was used while examining paired results of conservatively treated patients. According to Bioethical Commission of Poznan University of Science this research was not a medical experiment.

Results

Analysis revealed greater redisplacement during healing period in conservatively treated patients. Results proved to be significant in radial inclination and ulnar variance. Mechanism and morphology of fracture had an impact on the results. In group with extraarticular fractures (type A in AO classification) differences were clearer and significant in radial height, inclination and palmar tilt. In intraarticular group (type C in AO classification) parameters haven't varied so distinctly, and only ulnar variance differed significantly (**Figure 3, Table 3**).

Comparing redisplacement in conservatively treated patients, which occurred in particular periods of

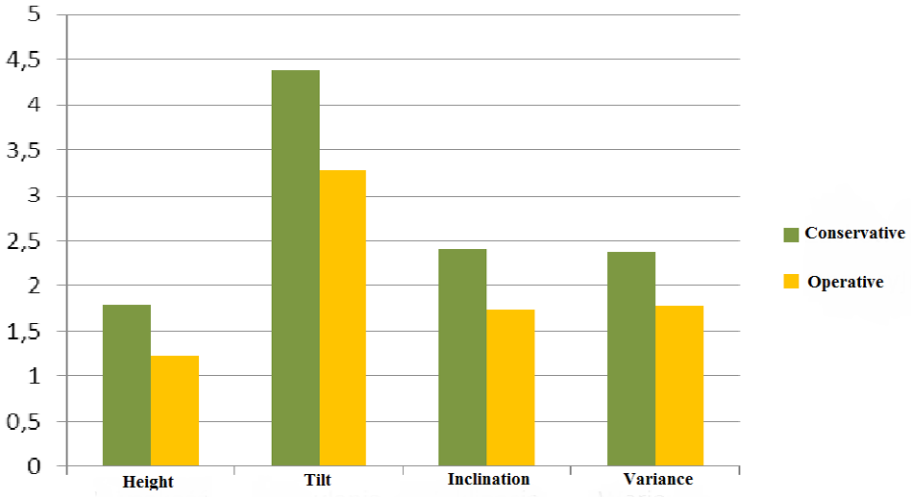

Figure 3. Average redisplacement between reposition and end of treatment in both groups

Table 3. Average redisplacement between reposition and end of treatment in both groups

	Height (mm)	Tilt (deg)	Inclination (mm)	Variance (deg)
Conservative	1.78	4.39	2.39	2.37
Operative	1.23	3.28	1.73	1.77
$p < 0.05$	ns	ns	0.02	0.03

immobilization revealed there is no significant difference between average change in radiological parameters in first 7 days and following weeks before cast removing.

Intraarticular fractures (type C) lead to greater redisplacement than extraarticular fractures (type A) irrespective to method of treatment. Differences were significant in ulnar variation (conservative treatment), radial inclination and palmar tilt (operative treatment).

Discussion

Among several radiological parameters describing three-dimensional structure of distal radius radial height, radial inclination, ulnar variance and palmar tilt were chosen. Posttraumatic changes of these parameters have greatest impact on biomechanical conditions and range of motion in wrist. AO classification was used to describe morphology of fractures, as it is one of the most accurate and enables to assess type of fracture in relatively objective manner by different doctors [11, 12]. Many physicians point out necessity of close control of healing process and fast diagnosing of redisplacement. However I haven't met uniform protocol of follow-up, 7 days intervals are considered as sufficient [13]. For patient's safety reason it would be appropriate to control fragments alignment every 7 days in first three weeks, when fracture consolidates [13]. Unfortunately, due to limited access to OP clinic X-ray scans were obtained after 7 days and 4 to 6 weeks in patients immobilized and after 6 weeks in operated patients. After 6 weeks risk of redisplacement is very low [14].

Examined group of elderly people, with average age close to 70, varied distinctly in respect of general condition, physical fitness, intellectual abilities and motivation to obey recommendation, as well as ability to finance further rehabilitation. For this reasons objective evaluation of functional results of treatment was impossible. Radically different needs and expectations make there is often no correlation between patient's satisfaction and objective, radiological outcome [15]. In this group there is no relation between anatomic reposition and functional results [16, 17]. Furthermore comparing dexterity of patients operated with volar plate, results showed, that older patients need almost 6 months longer rehabilitation to achieve progress similar to the young ones [18].

Analysis revealed greater redisplacement during healing period in conservatively treated patients in all parameters, but only radial inclination ulnar variance were significant. We have to consider different mechanism of trauma, leading to varied types of fracture. In type A fractures we often meet severe dorsal displacement and compression of dorsal cortex. In this group results were significantly better in all parameters except ulnar variance. However open reduction with internal fixation allows more accurate reposition, in type C fractures differences were not that clear. Main goal of this examination was to assess stability on reposition, not the quality. Therefore patients with worse reposition were less prone to redisplacement.

Two plates had to be removed, due to too long screws irritating extensor policis longus tendon. Luckily we avoided rupture of the tendon. No serious complication or infections were noticed in examined group.

Although conservative treatment should be reserved for nondisplaced and stable fractures, sometimes it must be conducted in patient, that don't agree for operation or are disqualified due to general condition. Each patient immobilized in cast underwent radiological control after 7 days. Immediate revealing of redisplacement and operative treatment gives better results than delayed osteotomies [21]. Evaluating redisplacement taking place in first 7 days and following weeks I found they don't vary significantly. Therefore we may implicate patients with borderline alignment of fragments should be qualified for surgery faster. Another closed reposition in these patients is difficult, and gives acceptable results in only 1/3 of all fractures [22].

Conclusions

Open reduction and internal fixation leads to minor redisplacement comparing to closed reduction and cast immobilization. Type of fracture has great impact on further changes of radiological parameters.

Intraarticular fractures (type C) lead to greater redisplacement than extraarticular fractures (type A) irrespective of method of treatment.

Redisplacement in operatively treated patients in first 7 days and following 4 to 6 weeks don't vary significantly.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Correspondence address:
Adam Zaręba
Kołobrzeska 10 C/7 Str.
78-400 Szczecinek, Poland
phone: +48 692274200
e-mail: zarebamd@gmail.com

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Ascertaining of temporomandibular disorders (TMD) with clinical and instrumental methods in the group of young adults

Anna Sójka[1], Juliusz Huber[2], Elżbieta Kaczmarek[3], Wiesław Hędzelek[1]

[1] Department of Prosthodontics, Poznan University of Medical Sciences, Poland
[2] Department of Pathophysiology of Locomotor Organs, Poznan University of Medical Sciences, Poland
[3] Department of Bioinformatics and Computational Biology, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. During the diagnostic process, the clinical examination of the masticatory system combined with jaw movement measurements can indicate the presence of temporomandibular disorders (TMD) symptoms.
Aim. The aim of the present study was to determine the presence of clinical symptoms of TMD in a group of subjects aged 19–27 years and to analyze the measurable parameters obtained from examinations carried out using the Arcus®digma.
Material and methods. Eighty four dentate subjects from 19 to 27 years were recruited from students. Objective signs were studied with Helkimo Anamnetic index (Ai) and the subjective symptoms were evaluated with Gsellmann's Occlusal Index (OI). Functional examination of the masticatory system was performed using Helkimo Clinical Dysfunction index (Di). Pain symptoms were determined in clinical examination using visual analogue scale. Software of Arcus®digma allowed the analysis of Bennett's angle and movement, the horizontal condylar inclination, retrusion, anterior guidance and Immediate Side Shift.
Results. Occasional pain occurred in 39% of students and correlated with increase of OI index, subjects with higher Di displayed higher values of OI. Comparison of Bennett's angle values for the right and left TMJ showed the significant asymmetry, similarly like the values of Bennett's movement and retrusion between the left and right TMJ.
Conclusions. Bennett's angle, Bennett's movement and retrusion were significant parameters in instrumental evaluation of TMD symptoms. The questionnaire and clinical examination supplemented with axiographic recordings revealed the presence of TMD symptoms in students who were not fully aware of TMJ disorders.

Keywords: temporomandibular disorders, clinical examination, axiography, measurable parameters.

Introduction

During the diagnostic process, jaw movement measurements combined with clinical examination of the masticatory system and the subjective report of pain can indicate the presence or absence of temporomandibular disorders symptoms. Clinical examination is the gold standard for diagnosing temporomandibular disorders (TMD) and involves assessment of jaw movements, acoustic symptoms, tenderness in jaw muscles and temporomandibular joints [1]. Condylar movements are not only determined by the pathologies in articular eminence, the disc and the ligaments of the temporomandibular joint but also by the occlusal and neuromuscular factors [2]. Axiography is a major part of instrumental analyses in clinical dental practices to evaluate functional states of the stomatognathic system. Recently, individual recording of mandibular movements has been made possible using the electronic jaw recording system. Patients with TMD frequently exhibit changes in the mandibular movements.

Electronic jaw recording systems (like Arcus®digma or Cadiax® systems) in patients with TMD display changes in patterns of jaw movements such as shortening or asymmetry in the condylar pathways. This is a noninvasive diagnostic method which allows the determination of the hinge axis as well as assessment of measurable parameters like Bennett's angle, the horizontal condylar inclination (HCI), shift angle, retrusion, incisal inclination during protrusion and laterotrusion and Immediate Side Shift (ISS). These parameters can be recorded for diagnostic purposes [3–5]. Few articles are available on the analysis of measurable parameters obtained from Arcus®digma and on their importance in the diagnosis of TMD.

The aim of present study was to determine the first symptoms of TMD in a group of subjects aged from 19 to 27 years, confirmed by the chosen measurable parameters obtained from examinations carried out using the Arcus®digma.

Material and methods

Eighty four dentate subjects (66 women and 18 men) aged from 19 to 27 years (mean 23 years ± 2 years) were recruited from university students to the study. They were recruited randomly and expressed a wish to check the functioning of the stomatognathic system. All they represented a similar body constitution and the anthropometric properties. Inclusion criteria were: good general health status without acute and chronic pain symptoms, full dentition, and stable occlusion, no prosthetic restorations either fixed or removable. Exclusion criteria were: former injury to the head and neck, dentofacial deformity or surgery of the temporomandibular joint (TMJ), TMJ trauma with chronic pain, concurrent systemic diseases, undergoing pharmacological therapy with drugs which might affect psycho-physical condition, orthodontic treatment. History was taken and an assessment form was filled. Those subjects whose treatment of TMD was expected before were excluded from the study.

Clinical studies

Pain sensation was evaluated by the Visual Analogy Scale (VAS) within the area of temporomandibular joint. Objective and subjective evaluation of signs was carried out. Objective signs were evaluated using the Helkimo Anamnetic Index (Ai) and the subjective symptoms with the Gsellmann's Occlusal Index (OI). Ai was scored based on the different symptoms of dysfunction in the masticatory system (subjective symptoms) reported by the individuals during history taking. Such index have three levels as follows: Ai-0 made up of individuals free from dysfunction symptoms, Ai-I, made up of individuals with mild dysfunction symptoms, Ai-II, made up of individuals with severe dysfunction symptoms. Functional examination of the masticatory organ using the Helkimo Clinical Dysfunction Index of TMD (Di) was performed. Helkimo Di = 0 (Di = 0) indicates no clinical signs of TMD, Helkimo Di = I (Di = I) mild signs, Helkimo Di = II (Di = II) moderate symptoms, Helkimo Di = III (Di = III) severe symptoms. In accordance with the presence and/or severity of clinical symptoms, individuals were assigned a score of 0, 1, or 5 points. The following symptoms could have been were observed: range of mandibular motion, TMJ function impairment, muscle tenderness during palpation, TMJ pain during palpation, pain during mandibular movement – only recorded when clearly identified. According to the score obtained, the subjects were classified in four groups: Di-0, 0 points – subjects clinically free from dysfunction symptoms, Di-I, 1 to 4 points – subjects with mild dysfunction symptoms, Di-II, 5 to 9 points – subjects with moderate dysfunction symptoms, Di-III, 10 to 25 points – subjects with severe dysfunction symptoms. The OI is based on 10 questions evaluating the subjects' symptoms within the stomatognathic system. Positive responses were graded as symptoms: 1 = light, 2 = medium, 3 = severe. The sum of the graded answers divided by the number of positive answers constitutes the Occlusal Index [3, 6, 7].

Axiographic recordings

Mandibular movements were recorded with an ultrasound based electronic Arcus®digma system (Arcus®digma, KaVo, Germany). During axiographic recording, the subject was seated in an upright position. First, the head frame with the receivers was attached. The maxillary position was determined with a bite plate, which had previously been individualized with hard silicone (EliteHD+Putty Zhermack). Next, a paraocclusal clutch was fixed with acrylic resin (Struktur 2 SC, VOCO) and was cemented with policarboxillic cement to the facial surfaces of the mandibular premolar and anterior teeth. Paraocclusal clutch did not interfere with the occlusion. The following tooth guided mandibular movements were recorded after initial instruction: maximum protrusive movement, maximum lateral movement to the right side, and maximum lateral movement to the left side. The measurements were processed with software allowing the calculation of the Bennett's angle, the horizontal condylar inclination,

individual mandibular side shift, the so-called Bennett's movement, retrusion, incisal inclination during protrusion and laterotrusion the so-called anterior guidance middle, left, right and Immediate Side Shift automatically. The measurable parameters values were referred statistically to the reference provided by Arcus®digma and to the examination results.

Ethical considerations were in agreement with the Helsinki Declaration. Approval was also received from the Bioethical Committee of University of Medical Sciences. Each subject was informed about the aim of study and gave written consent for examinations and data publication.

Statistical analysis

The sensation of pain rated as VAS scores, Helkimo Ai, OI, elicited from history and obtained from examinations are presented in the tables as counts (in absolute numbers) and as frequency (prevalence) expressed in percentage. The correlation of pain index (VAS scores and OI) with the clinical indexes was assessed using the Spearman rank correlation coefficient. Quantitative parameters of mandibular movements are presented in tables of mean values, standard deviations and minimal and maximal results. After evaluation of variance homogeneity results of these parameters were compared between left and right side using nonparametric paired Wilcoxon test. The results were considered as statistically significant at $p < 0.05$. Statistical analysis was performed using Statistica v. 9.0 (Stat Soft Inc).

Results

The VAS scores of 0 to 10 were applied to evaluate subjective and periodic pain and discomfort in the studied group of 84 subjects where 0 indicated no discomfort or pain. Such score was noted in 51 people (60.71%). Twenty three (27.38%) presented no subjective TMJ complaints Ai = 0, and 61 subjects (72.62%) reported slight subjective complaints Ai = I. The difference between the subjects reporting and not reporting subjective complaints was statistically significant ($p < 0.01$). In the studied group no severe complaints were reported Ai = II (**Table 1**). In the studied group Di = I (58.33%) was most common. Di = II was noted in significantly ($p < 0.01$) smaller number of people (23.81%). The clinical index Di = 0 was present in 17.86%, insignificantly different from Di = II (**Table 1**).

Subjective Occlusal Index by Gsellmann

In the studied group of 84 subjects, 14 (16.67%) reported no subjective complaints of TMJ dysfunction according to OI, 21 (25.00%) – showed a single subjective symptom what indicated a mild form of TMJ dysfunction, 31 (36.90%) – showed two symptoms indicating a medium dysfunction, and 18 (21.43%) – three or more symptoms which proved a severe form of TMJ dysfunction (**Table 2**).

Pain and dysfunction severity was evaluated in the students using three scales: VAS, OI, Di. It showed statistically significant positive correlations. The correlation rate between VAS and OI was 0.56 ($p < 0.05$), however between Di and OI was higher and showed the value of 0.82 ($p < 0.05$). An increase in VAS occurred with an increase in the OI index and the subjects with higher Di displayed higher values of OI (**Figure 1**).

Comparison of measurable parameters of the right and left TMJ

Measurable parameters of the TMJ mandibular movements obtained instrumental studies are presented in **Table 3**.

Comparing the values of the Bennett's angle for the right and left TMJ in the group of 84 subjects a statistically significant asymmetry at $p < 0.0001$ was found (**Figure 2**) as well as statistically significant asymmetry at $p < 0.0001$ for the values of Bennett movement between the left and right TMJ (**Figure 3**).

A statistically significant difference was found between retrusion parameters of the left (L) and right (R) TMJ at $p < 0.0001$ (**Figure 4**).

Table 1. Prevalence of TMDs according to anamnestic Helkimo index Ai and clinical Helkimo index Di

Anamnestic Helkimo index			Clinical Helkimo index		
Ai	Counts	%	Di	Counts	%
0	23	27.38	0	15	17.86
1	61	72.62	I	49	58.33
2	0	0	II	20	23.81
Total	84	100	Total	84	100

Table 2. Prevalence of TMDs according to Occlusal Index OI by Gsellmann

Occlusal Index (Gsellmann)		
OI	Counts	%
0	14	16.67
1	21	25.00
2	31	36.90
3	18	21.43

No statistically significant difference was found between the horizontal condylar inclination of the right and left TMJ condylar pathway in the studied subjects. The difference between the values of immediate side shift for the left and right TMJ is statistically non-significant. The anterior guidance parameters for the right (R) and left (L) TMJ do not show statistically significant differences.

Discussion

Properly elicited history and a detailed clinical examination are the basis for any diagnosis of disorders affecting the human body. Temporomandibular joints, due to their complicated structure and function, sometimes require a wider range of diagnostic methods including those which enable recording and visualizing individual mandibular movements. Certainly instrumental methods can supply much information; however this may give rise to some problems such as a noticeable changeability in the movement parameters of the masticatory system present both between patients and between subsequent measurements of the same patient. The data analysis poses problems, too. Determining the border movements depends on TMJ anatomy, occlusal plane, and physiological lim-

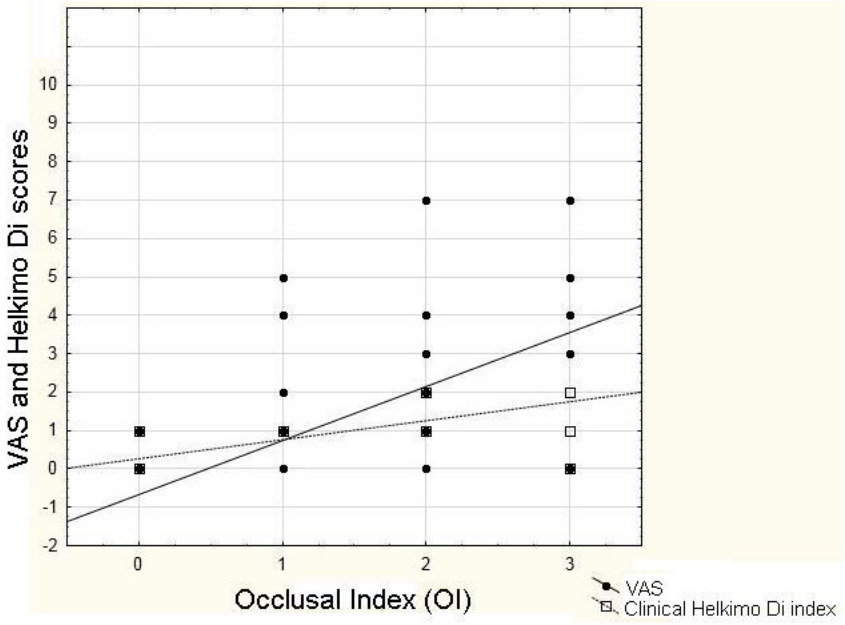

Figure 1. Correlation of OI and VAS ($r = 0.56$, $p < 0.05$) and clinical Helkimo Di index ($r = 0.82$, $p < 0.05$)

Table 3. Quantitative parameters of mandibular movements measured in a group of 84 subjects using Arcus®digma

Measurable parameters	Mean	Standard Deviation	Minimum	Maximum
Horizontal Condylar Inclination right TMJ (°)	34.57	12.35	0.00	63.30
Horizontal Condylar Inclination left TMJ (°)	36.47	11.68	0.00	63.90
Bennett angle right TMJ (°)	8.09	5.59	4.00	30.00
Bennett angle left TMJ (°)	12.28	7.19	4.00	30.00
Bennett movement right TMJ (°)	-8.62	14.81	-20.00	20.00
Bennett movement left TMJ (°)	4.08	15.92	-20.00	20.00
Retrusion right TMJ (mm)	0.93	0.83	0.00	3.50
Retrusion left TMJ (mm)	0.46	0.78	0.00	4.20
Immediate Side Shift right TMJ (mm)	0.01	0.04	0.00	0.30
Immediate Side Shift left TMJ (mm)	0.01	0.04	0.00	0.30
Anterior guidance middle (°)	40.16	18.19	0.00	66.90
Anterior guidance left (°)	41.69	16.96	-7.50	81.30
Anterior guidance right (°)	43.20	15.84	-5.00	69.60

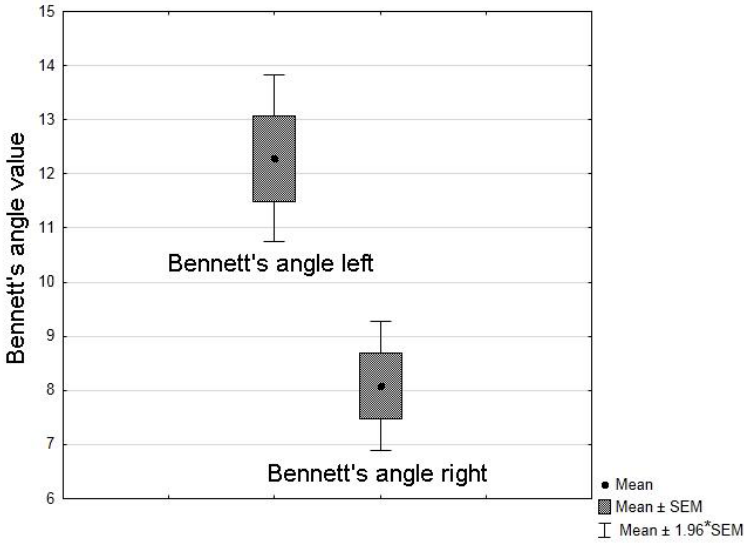

Figure 2. Bennett's angle in left and right TMJ ($p < 0.0001$)

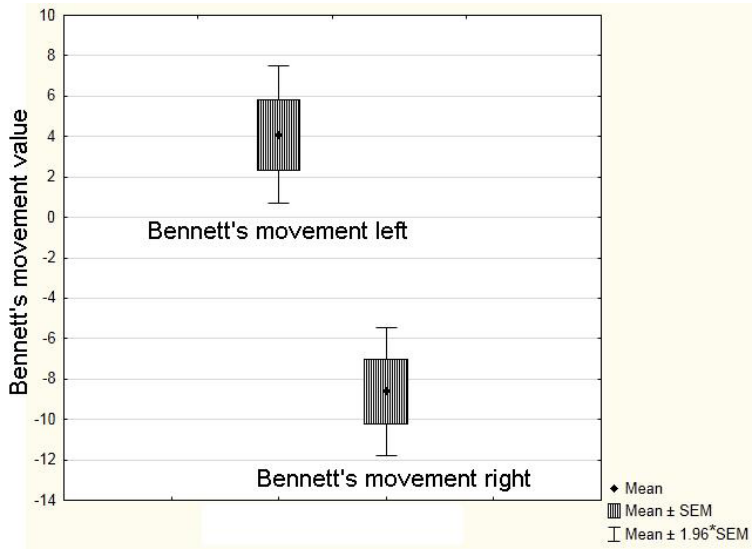

Figure 3. Bennett's movement in left and right TMJ ($p < 0.0001$)

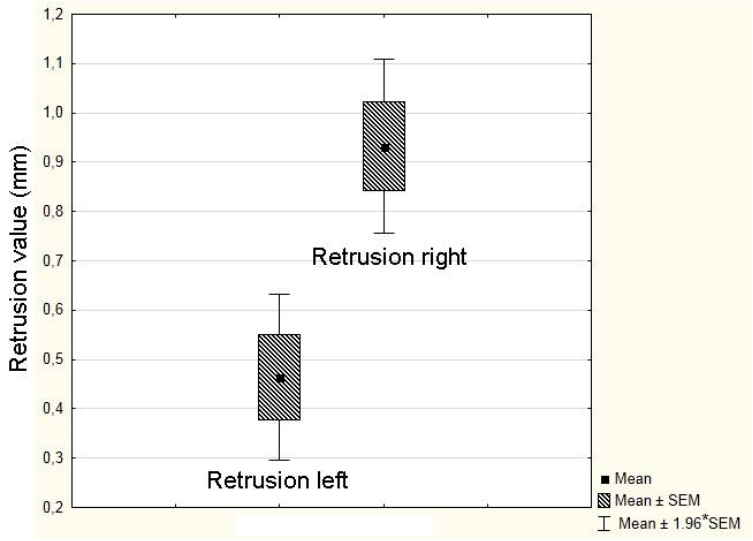

Figure 4. Retrusion of left and right TMJ ($p < 0.0001$)

its related to the ligaments system. The application of a paraocclusal tray in instrumental diagnostics enabled comparative studies of maximal intercuspidation and retrusive contact position.

Since we analyzed a group of students aged 19–27 years currently studying at medical university and other higher education schools, an initial problem appeared to be the choice of a group of fully healthy individuals. Therefore, the studied group of 84 subjects was analyzed without constructing a group of controls. Following history taking and physical examination, the groups of students with clinical symptoms of TMD were determined. However, following instrumental examination all results in various groups with particular dysfunctions were analyzed. In order to evaluate masticatory system function subjective indices were applied (Helkimo Ai and Gsellmann's Occlusal Index).

Clinical signs evaluation was done by Helkimo Di index. In the 1970's the author of these indices M. Helkimo basing on his observations noted major problems while evaluating various groups of patients [6, 7]. To facilitate comparison of these groups it was necessary to convert the results to numbers what would make the evaluation of symptoms frequency and severity easier. Since such an evaluation system was formulated by Helkimo, it has been commonly used for the evaluation of the masticatory system dysfunction. The subjective Gsellmann's Occlusal Index (OI) shows higher sensitivity as it requires answering 10 questions and may make an additional diagnostic element [3].

During clinical examination face asymmetry should be noted, which may result from previous occlusal or skeletal disorders. Muscular hypertrophy may also be observed due to teeth clenching or grinding or to TMJ and/or muscle inflammation oedema [8]. Asymmetry may also occur in patients with the history of facial nerve inflammation. The studies of Inui *et al.* revealed face asymmetry to be related to mandibular asymmetry; it is a common complaint of people with TMJ dysfunction [9]. The mandible may shift towards the affected side to avoid biting obstacles arising from condyle dislocation in the mandibular fossa.

It is believed that the evaluation of the masticatory system movement cannot be done using mechanical devices alone, but should also be accompanied by detailed clinical and imaging examinations. It should be remembered that the use of mechanical devices for evaluation is a functional analysis where the results repeatability are affected by many factors such as the condition of the neuro-muscular system, time of the day, psycho-physical state of a patient [10]. Baqaien *et al.* showed that horizontal condylar inclination is steeper with every millimeter of a protrusive movement as well as with age [11]. Female population has a tendency towards steeper values of HCI. However, in the study by Johnson and Winstanley, only 6 – 8% of the subjects showed the same HCI values [12]. In our study the average value of HCI for the right TMJ was 34.57°, for the left one 36.47° (Table 3). A high value of standard deviation (12.35 – right and 11.68 – left TMJ) suggests marked individual fluctuations of this parameter. However, no statistically significant difference was established between HCI of the right and left TMJ. Comparing the values of the Bennett's angle of the right and left TMJ in the group of 84 people a statistically significant asymmetry was found at $p < 0.0001$ (**Figure 2**). Average values of the Bennett movement parameter in the studied group for the right TMJ showed lateroretrusion of -8.62°, for the left TMJ lateroretrusion was 4.08° and standard deviation was 15.92 on average and displayed statistically significant asymmetry at $p < 0.0001$ between the right and left TMJ (**Table 3, Figure 3**). In the studied group ISS for the right and left TMJ was 0.01 mm and the difference between the right and left TMJ was not statistically significant. In our studies the anterior guidance was 40.16° and standard deviation was 18.19 and the difference was not statistically significant (**Table 3**). The statistical analysis of numeric values of measurable parameters in the group of 30 students studied by us before, showed a statistically significant difference for the Bennett movement parameter on the right and left side as well as for the retrusion parameter of the right and left side which could result from the left and right side asymmetry and from a TMJ disfunction. Tsuruta *et al.* showed greater shift from the maximum cuspidation to the reference position (central relation) in patients with condylar structural changes what may prove it is related to TMJ lesions [13]. An impact of retrusive movements on TMJ is unclear as in normal conditions the most retrusive mandibular position should be symmetrical on both sides. The temporomandibular joint may become compressed due to a retrusive movement of a working condyle, especially during parafunctions [14]. Results of instrumental recordings supplementing the results of clinical examination make possible to correctly diagnose the patient, ascertain the severity of TMD, give the basis for undertaking the implementation of directional prosthetic treatment.

Conclusions

While diagnosing TMDs it is recommended to apply both: the indices of subjective and clinical examinations. The questionnaire and the clinical examination revealed the presence symptoms of TMD in the majority of healthy students and they were not aware of the presence of TMJ disorders.

Comparing the results of measurable parameters in the group of 84 subjects, the statistically significant asymmetry was found between the right and left TMJ concerning the Bennett's angle, Bennett's movement and retrusion. This may prove the significance of these parameters in TMD diagnostics and may be useful in planning the future treatment. Examination by the Arcus®digma device can show both the symmetry as well as the asymmetry in TMJ function.

The analysis of measurable parameters and function charts obtained with the Arcus®digma device, together with the clinical findings, enables immediate evaluation of TMJ condition what makes it valuable in the diagnostics, prognosis and management of TMDs. Further studies are required concerning measurable parameters obtained with instrumental techniques and their application in TMD diagnostics.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Correspondence address:
Juliusz Huber
Department of Pathophysiology of Locomotor Organs
Poznan University of Medical Sciences
Wiktor Dega Clinical Orthopaedic and Rehabilitation Hospital
28 Czerwca 1956 135/147 Str.
61-545, Poznań, Poland
phone: +48 618310230
fax: +48 618310230
e-mail: zpnr@wp.pl

ORIGINAL PAPER

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Aborted myocardial infarction in patients undergoing primary percutaneous coronary intervention

Małgorzata Pyda[1], Stefan Grajek[1], Weronika Oleśkowska-Florek[2], Maciej Lesiak[1], Andrzej Siniawski[1], Adrian Gwizdała[1], Marek Grygier[1], Aleksander Araszkiewicz[1]

[1] 1st Department of Cardiology of the Poznan University of Medical Sciences, Poland
[2] Department of Cardiology of Public Specialized Health Care Center, Inowrocław, Poland

ABSTRACT

Introduction. The outcome of patients with ST-elevation myocardial infarction (STEMI) strongly depends on a successful reperfusion. In some patients receiving an effective treatment myocardial infarction can be aborted.
Aim. The aim of the study was to estimate the incidence, clinical outcome, prognosis and inflammatory response in patients with aborted MI.
Material and methods. 119 consecutive patients with STEMI treated with a primary percutaneous coronary intervention (pPCI) were enrolled in the study. Aborted MI was diagnosed when the maximal increase in cardiac enzymes (CK-MB) was up to twice the upper limit of normal (CK-MB ⩽ 50 U/l) and at least 50% reduction of ST-segment deviation was observed within 90 min of pPCI.
Results. Aborted MI was diagnosed in 16 subjects (13.4%). Patients with the aborted MI had lower serum troponin I levels ($p < 0.0001$). The time to treatment was significantly shorter in the aborted MI group (101 min vs. 220 min, $p < 0.00001$). Patients with aborted MI had a lower corrected TIMI frame count ($p < 0.05$) and a lower wall motion score index ($p < 0.005$), less pronounced inflammatory response (lower serum levels of IL-6, $p < 0.01$, and MCP-1, $p < 0.05$), higher ejection fraction six months after MI (72% vs. 64%, $p < 0.05$). None of the aborted MI patients died during the 3-year follow-up, while there were 13 deaths among patients with non-aborted MI.
Conclusions. The abortion of myocardial infarction results in a better outcome and more favorable prognosis. An inflammatory response is less pronounced in the aborted MI.

Keywords: STEMI, primary PCI, aborted MI.

Introduction

Myocardial infarction (MI) being the most dramatic manifestation of coronary artery disease leads to heart failure, other severe complications and is still associated with high mortality. The currently published ESC and ACC/AHA guidelines on ST-elevation myocardial infarction (STEMI) management show a great progress in the treatment of acute MI in the recent years [1, 2].

Immediate diagnosis and treatment in specialized centers may improve outcome. It is believed that some of the patients who have undergone successful reperfusion therapy will avoid myocardial necrosis [3]. This phenomenon, referred to as "aborted myocardial infarction", was first described in the Myocardial Infarction Triage and Intervention study (MITI) in subjects who received a very early treatment not followed by a raise of MI biomarkers [4]. It showed that 40% of patients treated within 3 hours of symptoms had no evidence of infarction as measured by thallium scanning and 35% had minimal infarct size < 10% of the left ventricle. This population was further evaluated by Lamfers et al. in a study comprising 42 patients [5]. He

defined aborted MI as both, a raise of cardiac enzymes (CPK or CK-MB) not higher than twice the upper limit of normal, and reduction of ST-segment deviation greater than or equal to 50%. In the clinical practice myocardial necrosis is usually confirmed and quantified based on the serum cardiac biomarker levels. However, it is the troponin concentration which is considered the most sensitive marker of myocardial necrosis, and thus a definition of aborted MI becomes more complex. Therefore Hassan et al. suggested troponin T level of 1.5 µg/L as a cut-off value for aborted MI [6].

The assessment of resolution of initial ST-segment elevation in a standard 12-lead ECG is still considered to be a simple method of measuring myocardial perfusion and is therefore used for definition of aborted MI. Moreover, it allows for the determination of mortality risk in STEMI [7, 8].

Material and methods

The study protocol of the research was approved by our University Ethical Committee and all subjects gave informed consent to the work. Between January 2009 and December 2010 at the 1st Department of Cardiology of the Poznań University of Medical Sciences we diagnosed 1800 cases of ACS, among which 750 were nSTEMI, 1050 – STEMI, 150 – referred to cardiac surgery, and 900 were treated with pPCI. Only 119 patients with STEMI (82 men (69%), mean age 59 ± 10 years) presented with the first onset of symptom lasting between 30 minutes and 6 hours. They were enrolled in the study and divided into two groups: non-aborted MI and aborted MI. Aborted MI was diagnosed when the maximal increase in cardiac enzymes (CK-MB or CPK) was smaller than or equal to twice the upper limit of normal and resolution of ST-segment deviation was greater than or equal to 50% within 90 min of pPCI (index procedure).

Of the total cohort of 119 patients, 16 (13.4%) met the criteria of aborted MI. **Table 1** shows the basic clinical characteristics.

STEMI was diagnosed when ST segment elevation measured at J point was ⩾ 0.1 mV in at least two consecutive leads or ⩾ 0.2 mV in two adjacent precordial leads in a standard 12-lead ECG. The basic clinical and laboratory data (including total cholesterol, HDL and LDL cholesterol, triglycerides, leukocytes, creatinine, glucose, ESR, CPK, CK-MB and cTnI, interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and CRP levels were analyzed.

Blood samples were collected before the index procedure as well as 4, 8, 12, 16, 24 and 36 hours of hospital arrival. A 12-lead ECG was recorded before and 90 minutes after the index procedure. In cases of anterior MI, the sum of ST-segment elevation in leads V1 to V6, I, and aVL was added to the sum of ST-segment depression in leads II, III, and aVF. For inferior MI, the sum of ST-segment elevation in leads II, III, and aVF (and I, aVL, V5, and V6, if present) was added to the sum of ST-segment depression in leads V1 to V4 [3, 4]. The resolution of all ST-segment deviation was calculated based on the baseline ECG and the one recorded 90 minutes post pPCI. The corrected TIMI Frame Count (CTFC) method was used for evaluation of flow in the infarct related artery [9].

An echocardiography was performed on the 2nd or 3rd day of hospitalization and 6 months after an ACS episode. The left ventricular (LV) end-diastolic volume

Table 1. Baseline characteristics of study population

		Aborted MI n = 16	Non-aborted MI n = 103	p
Sex	Female	9	28	0.0407
	Male	7	75	
Age (years)*		56 ± 6	60 ± 11	0.2237
BMI (kg/m2)*		26.1 ± 5.3	26.1 ± 3.5	0.9886
Arterial hypertension		13(81%)	43(42%)	0.0075
Diabetes		0(0%)	15(15%)	0.2194
Hypercholesterolemia		16(100%)	79(77%)	0.0678
Tobacco smoking		12(75%)	58(56%)	0.2542
Positive family history		6(38%)	29(28%)	0.6396
Previous stroke		0(0%)	3(3%)	0.8684
Peripheral arterial disease		0(0%)	11(11%)	0.3637
Time to treatment (min)*		101.3 ± 34.4	220.7 ± 65.1	0.00001
Killip-Kimball class > 1		0(0%)	8(8%)	0.5368

**value ± standard deviation*

(EDV), end-systolic volume (ESV), ejection fraction (EF) and left ventricular mass index (LVMI) were evaluated, wall motion score index (WMSI) was measured using the 4-grade wall motion score: 1 – normokinesis, 2 – hypokinesis, 3 – akinesis, 4 – dyskinesis involving 17 segments of LV. The risk was stratified in all patients using the TIMI Risk Score by Morrow and Antman [10].

Statistical analysis

The normality of distribution of the analyzed variables was verified using the Kolmogorov-Smirnov test (K-S test). If a normal distribution was rejected, medians and extreme values were used. Normally distributed variables were presented as arithmetic mean ± standard deviation. Results were found statistically significant if p value scored < 0.05. The T-student test was used to verify the statistical significance of differences between values showing a normal distribution; otherwise, the Mann-Whitney test was used. Qualitative characteristics were presented as a percentage (%) of the observed cases. The Chi-square test was used to analyze the distribution differences between those variables.

The analyses of variance for repeatable measurements were carried out using the least significant difference (LSD) test for the variables that were observed on two separate occasions (EF, EDV, ESV). Discriminating and logistic regression analyses were also performed.

Results

The subjects with aborted MI were significantly more often female and hypertensive (p < 0.05). Age, BMI, diabetes mellitus, hypercholesterolemia, family history, tobacco smoking and Killip class were similar in both groups. The time to treatment was significantly shorter in patients with aborted MI (101.3 ± 34.4 vs. 220.7 ± 65.1, p < 0.00001).

There were no significant differences between the two groups regarding the levels of total cholesterol, HDL and LDL cholesterol, triglycerides, leukocytes, glucose, creatinine, and erythrocyte sedimentation rate (ESR) (**Table 2**). The serum levels of IL-6 and MCP-1 before the reperfusion therapy were higher in the group of non-aborted MI. No significant difference was found between the two groups in serum CRP level before pPCI.

No difference was found between the groups in the incidence of a single- or multi-vessel coronary heart disease. However, a single-vessel disease tended to be more frequent in subjects with aborted MI (75% vs. 48%, p = 0.07). The aborted MI patients had also a significantly better flow in the infarct related artery after the index procedure as measured with the corrected TIMI Frame Count (**Table 3**). Moreover, they had a significantly lower maximal ST-segment elevation 90 minutes after the procedure and a greater reduction of the maximal ST-segment elevation. In the non-aborted MI group, a pathological Q wave was observed significantly more often (p < 0.05). The study groups did not differ in terms of MI location, number of ST elevation leads, persistent ST segment elevation and heart rate (**Table 4**).

The echocardiographic parameters are summarized in **Table 5**. A follow-up examination at 6 months showed higher EF and lower EDV and ESV in the abort-

Table 2. Results of laboratory tests

	Aborted MI n = 16	Non-aborted MI n = 103	p
Total cholesterol (mmol/l)*	7.1 ± 1.3	6.1 ± 1.4	0.0800
LDL (mmol/l)*	4.5 ± 1.3	4.0 ± 1.4	0.2948
HDL (mmol/l)*	1.7 ± 0.7	1.4 ± 0.4	0.1127
Triglicerydes (mmol/l)*	1.9 ± 0.9	1.7 ± 0.9	0.6080
Leukocytes (nG/l)*	10.4 ± 2.9	11.2 ± 4.1	0.6243
Glucose(mmol/l)*	6.2 ± 1.2	7.2 ± 3.9	0.4347
ESR (mm/h)*	11.8 ± 6.8	15.7 ± 11.1	0.3212
Creatinine (µmol/l)*	77.2 ± 13.7	87.7 ± 22.1	0.1917
Troponin I (ng/ml)*	1.6 ± 2.0	18.2 ± 18.0	0.00006
CPK (U/l)*	202.1 ± 234.0	2346.0 ± 1813.2	0.00002
CK-MB (U/l)*	28.4 ± 14.2	206.4 ± 154.3	0.00002
IL-6 (pg/ml)*	0.8 ± 1.4	19.9 ± 54.4	0.0071
MCP-1 (pg/ml)*	130.0 ± 123.2	216.9 ± 143.2	0.0232
CRP (µg/ml)*	4.4 ± 4.3	8.4 ± 17.8	0.7111

**value ± standard deviation*

Table 3. Angiographic data in study population

	Aborted MI n = 16	Non-aborted MI n = 103	P
Single vessel disease	12 (75%)	49 (48%)	0.0762
Multi vessel disease	4 (25%)	54 (52%)	
CTFC (number of frames)*	19.9 ± 2.2	36.1 ± 30.0	0.0080

**value ± standard deviation*

Table 4. Electrocardiographic data of study population

	Aborted MI n = 16	Non-aborted MI n = 103	p
Location of MI:			
anterior	9 (56%)	47 (46%)	
inferior	7 (43%)	49 (47%)	0.3041
lateral	0 (0%)	7 (7%)	
Number of leads with ST elevation*	4.1 ± 1.5	4.0 ± 1.8	0.8732
Max. ST elevation (mm)*:			
at admission	2.9 ± 1.9	3.2 ± 1.6	0.2371
90 minutes after PCI	0.4 ± 0.5	1.8 ± 1.2	0.000002
Reduction of max. ST elevation (%)*	83.8 ± 22.4	43.9 ± 31.3	0.00001
Persistent Q wave	5 (31%)	72 (70%)	0.0064
Persistent ST elevation	0 (0%)	22 (21%)	0.0549
Heart rate (/min)*	70.0 ± 10.5	78.1 ± 16.9	0.0642

**value ± standard deviation*

Table 5. Echocardiographic parameters during hospitalization and 6 months after MI in study population

	Aborted MI n = 16	Non-aborted MI n = 103	p
EF-1 (%)*	62.1 ± 7.7	55.5 ± 12.7	0.0766
EF-2 after 6 months (%)*	72.0 ± 4.5	64.0 ± 12.9	0.0137
EDV-1 (ml)*	69.5 ± 32.4	96.7 ± 35.7	0.0469
EDV-2 after 6 months (ml)*	61.0 ± 23.3	90.4 ± 37.1	0.0259
ESV-1 (ml)*	25.5 ± 13.2	43.5 ± 21.0	0.0310
ESV-2 after 6 months (ml)*	15.4 ± 7.4	35.9 ± 24.7	0.0119
WMSI *	1.1 ± 0.1	1.3 ± 0.3	0.0048
LV mass index-1*	190.9 ± 46.1	269.7 ± 281.9	0.0147
LV mass index-2 after 6 months*	177.6 ± 47.1	257.1 ± 282.2	0.0161

**value ± standard deviation*

ed MI patients. Both, WMSI and LVMI, were lower during the hospitalization (LVMI-1) as well as in the follow-up examination (LVMI-2). The TIMI Risk Score was similar in both groups (2p vs. 2p).

Thirteen (12.6%) out of 103 non-aborted MI patients and none of the aborted MI group died during the 3-year follow-up. Due to a relatively small and unbalanced number of patients in each group, the Kaplan-Meier survival curve in **Figure 1** failed to show a significant difference between the studied groups.

Discussion

We diagnosed aborted MI when: (i) a patient suffered a typical chest pain for over 30 min; and (ii) the maximal increase in cardiac enzymes (CK-MB or CPK) was smaller than or equal to twice the upper limit of normal; and (iii) the resolution of the initial ST-segment deviation was greater than or equal to 50% [5]; and (iv) there was a significant coronary artery stenosis at the territory of suspected ischemia. In our study the aborted MI patients accounted for 13.4% which is a similar result (15%) to the one reported by Sciagra et al. [11], Hassan et al. (18%) [6] and Vasile (9.1%) [12].

In 2012 the European Society of Cardiology proposed the third universal definition of myocardial infarction based, among others, on a very sensitive marker – troponin; however the definition of aborted MI was not included [13]. Following the MI definition, all of the studied patients had elevated troponin lev-

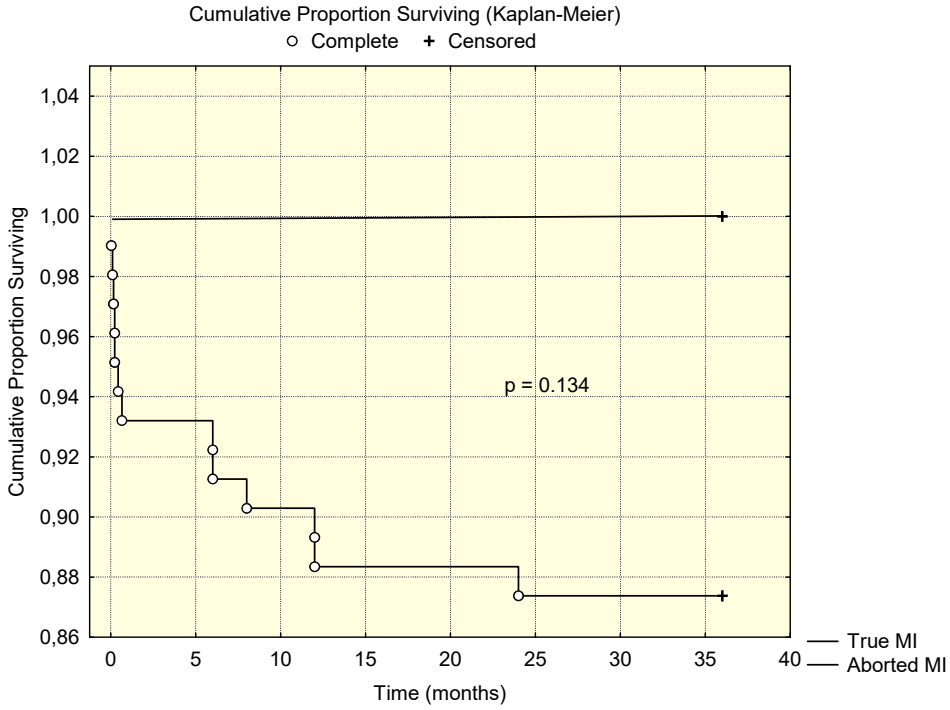

Figure 1. Kaplan-Meier Curves for both groups of study population

els. The frequency of aborted MI would be 0% when using 99th percentile cut-off of troponin level. The time to treatment in the studied population was never shorter than 60 minutes. This is probably why all of our studied patients experienced myocardial necrosis reflected by elevated troponin levels. However, as mentioned earlier, troponin is a very sensitive marker allowing detection of even minor myocardial damage involving a small myocyte count. Consequently, a question arises if it would be at all feasible for clinical practice to implement a definition of aborted MI based on the detection of elevated troponin levels. This issue remains controversial. In a study by Vasile at al. the mean troponin T level in aborted MI was 0.82 ± 1.82 vs. 5.64 ± 6.18 µg/l in the entire study population [12].

It seems that similar concern arose in Hassan's group when suggesting a troponin cut-off point for their definition of aborted MI. They included into the aborted MI group certain patients. The discriminating criteria were troponin T < 1.5 µg/L and CK release up to 3-fold the upper limit of normal [6]. Therefore, this approach allowed to identify patients with a more favorable outcome. It is commonly recognized that pPCI itself is associated with cardiac biomarker release. Similarly,in our study the mean troponin level in the aborted MI group was also 1.6 ± 2.0 µg/L [3] while being significantly higher in the non-aborted MI subjects (18.2 ± 18.0 µg/L).

In present study, time to treatment was significantly shorter in the aborted MI patients, which is consistent with other reports [3, 11]. This further confirms that time is an important factor influencing the extension of necrosis and mortality in patients with STEMI treated with pPCI [14, 15].

We also observed, as shown in other studies, that subjects with aborted myocardial infarction had better flow in the infarct related artery (corrected TIMI Frame Count) [16–18].

Antiplatelet therapy involving GP IIb/IIIa inhibitors significantly improves myocardial perfusion and limits necrosis [19].

To date, our study first examined the inflammatory reaction following a primary PCI in patients with aborted MI. Less pronounced inflammatory response is an important finding, not reported previously in any studies concerning aborted MI. It translates to a smaller area of myocardial necrosis and myocardial scarring and remodeling of the left ventricle [20, 21]. Our study documented that patients with aborted MI had lower plasma levels of both, IL-6 and MCP-1 (**Table 2**). Higher MCP-1 and IL-6 plasma levels have been demonstrated to correlate with increased risk of death and outcome in patients with acute coronary syndrome [22–24].

We have also observed that patients with aborted MI had a better left ventricular function, which significantly improves the prognosis of infarct survivors [25–27].

In their paper, Eitel at al. showed that 56% of patients with aborted MI had no scar detected while 44% of subjects had only minor necrosis in MRI study [28]. It may implicate limited unfavorable remodeling and better prognosis.

The pathological Q wave was more frequently observed in the non-aborted MI patients (**Table 4**). Usually, the presence of pathological Q wave is an electrocardiographic evidence that more than a half of the wall thickness is affected by a scar. As an electrocardiographic sign, the Q wave however not always truly reflects the size of necrosis [29]. On the other hand, the absence of Q wave is an excellent predictor of left ventricular function recovery following a successful reperfusion therapy [30].

The definition of aborted MI used in our study seems justified, as patients with aborted MI had better prognosis which manifested itself in only slightly impaired left ventricular function.

Based on our results, we can conclude that the definition of aborted MI after pPCI should be revised to account for the troponin criteria proposed by Hassan [6]. Whether patients with aborted MI should be administered the same treatment for the secondary prevention as those with non-aborted MI is still an open issue.

Our study was a retrospective analysis comprising a relatively small group of patients which may be its limitation. Nevertheless, our results documented that abortion of myocardial infarction by prompt reperfusion remains an essential target for PCI.

Conclusions

The abortion of myocardial infarction by means of a fast and efficient reperfusion therapy results in a better clinical outcome and more favourable prognosis. The inflammatory response is less pronounced in subjects with aborted MI. Therefore, the abortion of MI should become a target in the acute treatment of myocardial infarction.

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Correspondence address:
Małgorzata Pyda
1st Department of Cardiology
1/2 Długa Str, 61-848 Poznań, Poland
e-mail: nikaflorek@gmail.com
phone: +48 509105925
fax: +48 618549537

ORIGINAL PAPER

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Soluble selectins and highly fucosylated α1-antichymotrypsin in rheumatoid arthritis patients

Anna Olewicz-Gawlik, Izabela Korczowska-Łącka, Paweł Hrycaj

Department of Rheumatology and Clinical Immunology, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. Fucosylation of acute phase proteins and serum soluble selectin levels is increased in rheumatoid arthritis (RA) patients and can influence leukocyte extravasation.
Aim. The aim of this study was to evaluate the concentration and fucosylation of α1-antichymotrypsin (ACT) in relation to serum concentrations of soluble forms of selectins in RA patients.
Material and methods. Serum samples of 70 RA patients and 30 healthy controls were examined using sandwich enzyme-linked immunosorbent assay (ELISA).
Results. ACT-FR was significantly increased in RA patients when compared to healthy controls ($p < 0.001$) and significantly correlated with serum concentrations of rheumatoid factor (RF) and antibodies against cyclic citrullinated peptides (ACPA) ($p = 0.006$, $p = 0.04$, respectively). Moreover, we found significant correlations between the serum levels of soluble (s)P- and sE-selectin and ACT-FR ($p = 0.008$ and $p = 0.03$, respectively) only in male RA patients.
Conclusions. Fucosylation of ACT differs between male and female RA patients and is related to sP- and sE-selectin levels only in men.

Keywords: acute phase proteins, fucosylation, rheumatoid arthritis, selectins.

Introduction

Rheumatoid arthritis (RA) is a systemic, autoimmune inflammatory disease of unknown origin [1]. The hallmark of RA is a chronic inflammation of joints resulting in joint destruction, functional disability and increased mortality of patients [2]. Systemic inflammation, present in majority of RA patients and reflected in symptoms and biochemical tests, is characterized by activation of vascular endothelium, leukocytes and platelets. These activated cells express selectins [3], which play important roles in coordination of leukocyte migration to extravascular sites of inflammation [4]. E-selectin is expressed only by endothelial cell and circulating E-selectin reflects endothelial activation [5] and mediates angiogenesis via its endothelial ligand sialyl LewisX (sLe^x) [6]. P-selectin is an adhesion molecule located in the membrane of alpha granules of platelets and in the Weibel-Palade bodies of endothelial cells, and its soluble (s) form is a clinical marker of platelet activation [5]. L-selectin is expressed by granulocytes, lymphocytes and monocytes and sL-selectin is a regulator of leukocyte attachment to endothelium [7]. Soluble forms of selectins can be detected in circulation, and their raised levels have been reported in RA [8]. All selectins recognize sialylated, fucosylated glycoconjugates expressed on glycoproteins [9]. Recent outcomes emphasized a critical role of post-translational modifications such as glycosylation in the pathophysiology of many autoimmune diseases. Increased fucosylation of many serum proteins, including antibodies against cyclic citrullinated peptides (ACPA), have been observed in RA patients [10, 11]. The majority of the fucose residues were detected as sLe^x on α1-acid glycoprotein (AGP), α1-antichymotrypsin (ACT) and haptoglobin [12].

ACT is a serine protease inhibitor which rises in inflammatory conditions and has been implicated in the pathology of a number of devastating human diseases including chronic obstructive pulmonary disease, Parkinson's disease and Alzheimer's disease [13]. In RA the serum concentration of ACT was described as a reliable indicator of the mass of inflamed tissue [14]. ACT acts as an inhibitor of leukocyte cathepsin G, which is involved in the pathophysiology of RA [15] and has the unique ability amongst serpins to bind to DNA [16].

As sLe^x epitope on fucosylated glycoproteins can act as a ligand for both membrane bound selectins and their circulating forms, fucosylated proteins can interact with soluble selectins and influence their function. In this study we assessed concentration and fucosylation of ACT in relation to serum concentrations of soluble forms of selectins and disease activity in RA patients.

Material and methods

Patients

We recruited to the pilot study 70 consecutive patients (52 women, 18 men, age range 25–75 years, mean 54.7 ± 12.6 years), all of whom fulfilled the 2010 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) criteria for RA [17]. Patients were also grouped as having early RA (n = 16) or established RA (n = 54), based upon the duration of the disease, defining early RA as patients having a disease duration of less than 2 years and established RA as patients having a disease duration longer than 2 years. Informed consent was obtained from all participants and the study was approved by the Institutional Review Board at Poznan University of Medical Sciences. A protocol of the conducted research conforms to the principles of the World Medical Association's Declaration of Helsinki.

Clinical evaluation and laboratory measurements

The workup included the demographics of patients, patients' history, a thorough clinical examination together with patients' status/disease activity assessment using Disease Activity Score (DAS 28) [18, 19]. Functional status was evaluated with Polish version of the Health Assessment Questionnaire (HAQ) disability index [20]. The patients were also asked to assess their overall activity of arthritis and pain using visual analog scales (VAS) [21]. In addition, the 66/68-joint count was performed (66 peripheral joints were evaluated for swelling and 68 peripheral joints were evaluated for tenderness or pain on motion).

Sera from patients were collected at the time of clinical examination and stored at -70°C before estimations were made. The control sera from 30 healthy blood donors (20 women, 10 men, age groups: 25–34 years, 35–44 years, 45–54 years, 55–65 years) were purchased from Regional Blood Center to be analyzed individually. The samples were also pooled and stored at -70°C to be used as a normal control in the lectin enzyme-linked immunosorbent assay (ELISA). Erythrocyte sedimentation rate (ESR) was determined by routine method. Serum C-reactive protein (CRP), AGP and ACT concentrations were measured by rocket immunoelectrophoresis [22]. The serum sL-, sP-, sE-selectin levels and the concentrations of IgM rheumatoid factor (RF) and ACPA were measured using commercially available ELISA kits (R&D Systems, USA for selectins and Euroimmun, Germany for IgM RF and ACPA), according to the manufacturer's instructions. The fucosylation of ACT were determined by the Aleuria aurantia lectin (AAL) ELISA, as described previously [23]. The fucosylation ratio (ACT-FR) was calculated as the ratio of the mean sample absorbance to the mean absorbance of the normal pool, after blank subtraction. All samples were assessed in duplicate.

Statistical analysis

Patients' demographic data were analyzed using descriptive statistics. All continuous data were tested for normal distribution using the Kolmogorov-Smirnov test. The results of analysis of normally distributed data were presented as mean ± standard deviation (SD), whereas non-normally distributed data were expressed as median (interquartile range, IQR). Differences between investigated groups were tested for significance using a Mann-Whitney U-test. Correlations between variables within the group were analyzed using Spearman's rank-order correlation coefficient (r). Results were considered significant at $p < 0.05$ two-sided. All statistical analyses were performed with STATISTICA software (Statsoft, 2009. Statistica version 9.1, www.statsoft.com).

Results

Table 1 shows the characteristics of RA patients.

Sixty four (91.4%) patients received disease-modifying anti-rheumatic drugs (DMARDs): 48 (68.6%) patients received methotrexate, 12 (17.1%) were treated with sulphasalazine, 3 (4.3%) with leflunomide and 1 (1.4%) with chloroquine. Forty two (60%) patients

were treated with glucocorticoids (daily mean dose equivalent to prednisone was 5.6 mg), from whom 30 received both glucocorticoids and methotrexate. Two women were taking oral contraceptives at the time of examination.

Fucosylation of ACT was significantly lower in healthy controls when compared to RA patients (0.9(0.5) vs. 1.5(1.9), $p < 0.001$, **Figure 1**). It was not associated with age or disease duration of the investigated subjects. In RA patients ACT-FR was significantly higher in men than in women ($p = 0.01$, **Figure 2**). ACT-FR significantly correlated with serum concentrations of RF and ACPA ($r = 0.32$, $p = 0.006$ and $r = 0.25$, $p = 0.04$, respectively). The correlation between ACT-FR and ACPA was more significant in the group of RA patients not treated with glucocorticoids

Table 1. Demographic, clinical and laboratory characteristics of rheumatoid arthritis patients

Sex (women/men)	52/18
Age (years), mean (range)	54.7 ± 12.6 (25–75)
Duration of the disease (years), mean (range)	10.5 ± 11.4 (0,5–56)
IgM rheumatoid factor present, n (%)	60 (85.7)
anti-cyclic citrullinated peptide antibodies present, n (%)	48 (68.6)
Health Assessment Questionnaire disability index	1.4 (0.9)
Visual analog scale pain, mm	46.5 (32)
DAS 28 (4 variables)	5.3 (1.9)
DAS 28 (3 variables)	5.2 (1.9)
Number of tender joints	21 (24.5)
Number of swollen joints	12 (11.5)
Erythrocyte sedimentation rate, mm/h	20 (35)
C-reactive protein, mg/l	3 (9.2)
sE-selectin (ng/ml)	24.7 (16.9)
sP-selectin (ng/ml)	47.3 (63)
sL-selectin (ng/ml)	1909.1 (1022.5)
α1-antichymotrypsin fucosylation ratio (ACT-FR)	1.5 (1.9)

Data presented as the median (interquartile range), mean ± standard deviation or as n (%)

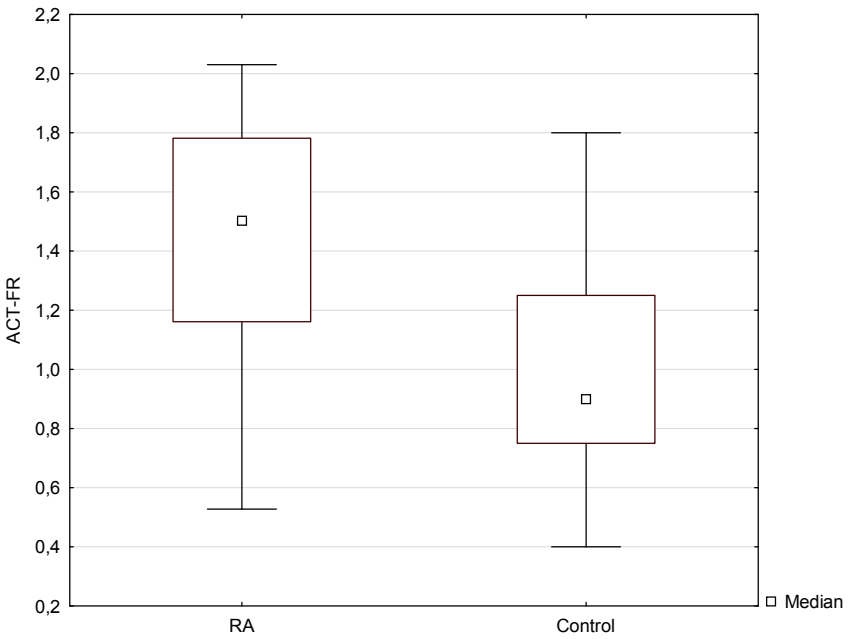

Figure 1. α1-Antichymotrypsin fucosylation ratio (ACT-FR) in rheumatoid arthritis (RA) patients and healthy control (Control)

(r = 0.46, p = 0.01). Moreover, ACT-FR and sE-selectin levels were significantly lower in patients with negative RF vs. positive RF (p = 0.02). We noted statistically significant correlation of ACT-FR with sP-selectin (r = 0.6, p = 0.008, **Figure 3**) and sE-selectin (r = 0.5, p = 0.03, **Figure 4**) only in males with RA. No correlation was observed between ACT-FR and sL-selectin level.

No other statistically significant associations were detected between ACT-FR and clinical or laboratory findings, as shown in Table 1.We also noted no statistically significant differences in ACT-FR and soluble selectin levels between the group with early vs. established RA or between patients undergoing different immunosuppressive therapies.

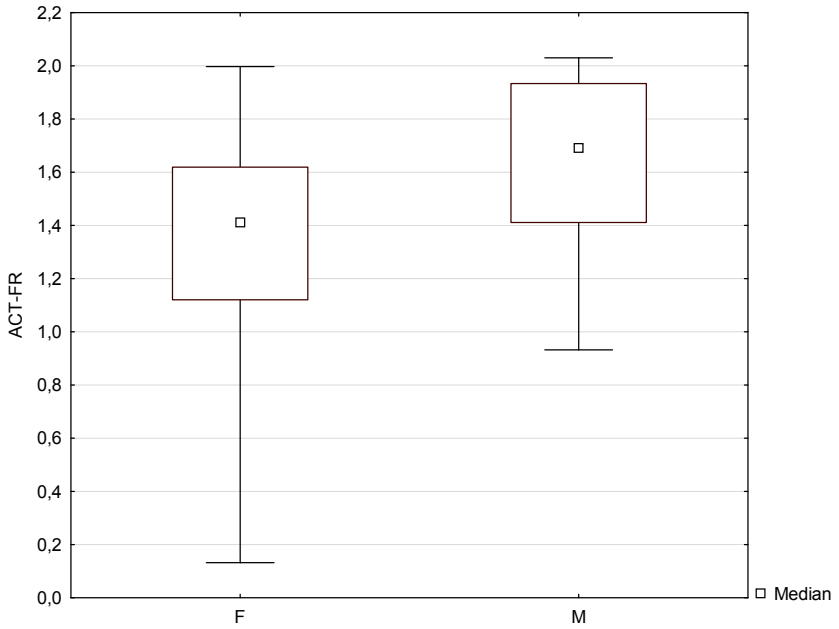

Figure 2. α1-Antichymotrypsin fucosylation ratio (ACT-FR) in female (F) and male (M) rheumatoid arthritis patients

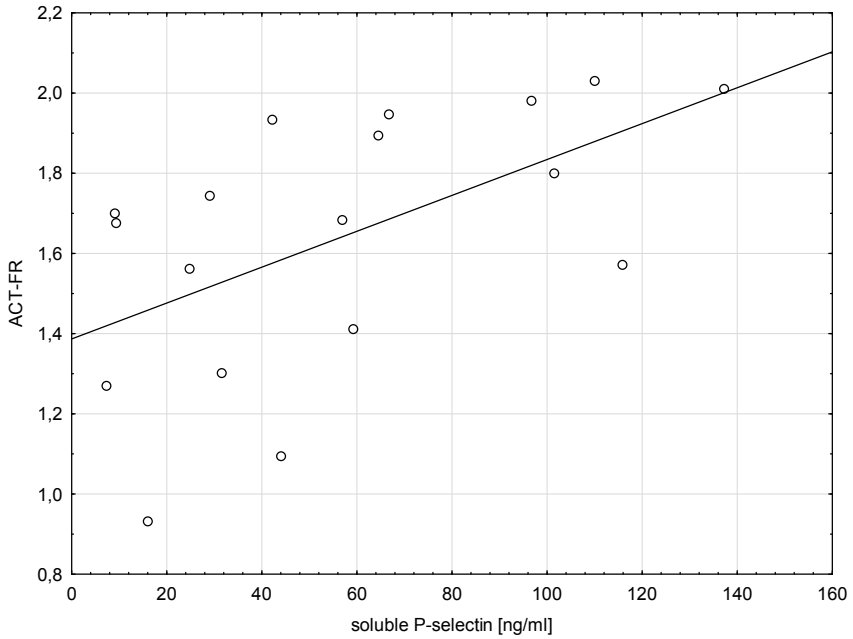

Figure 3. Correlation between α1-antichymotrypsin fucosylation ratio (ACT-FR) and soluble P-selectin in male patients with rheumatoid arthritis

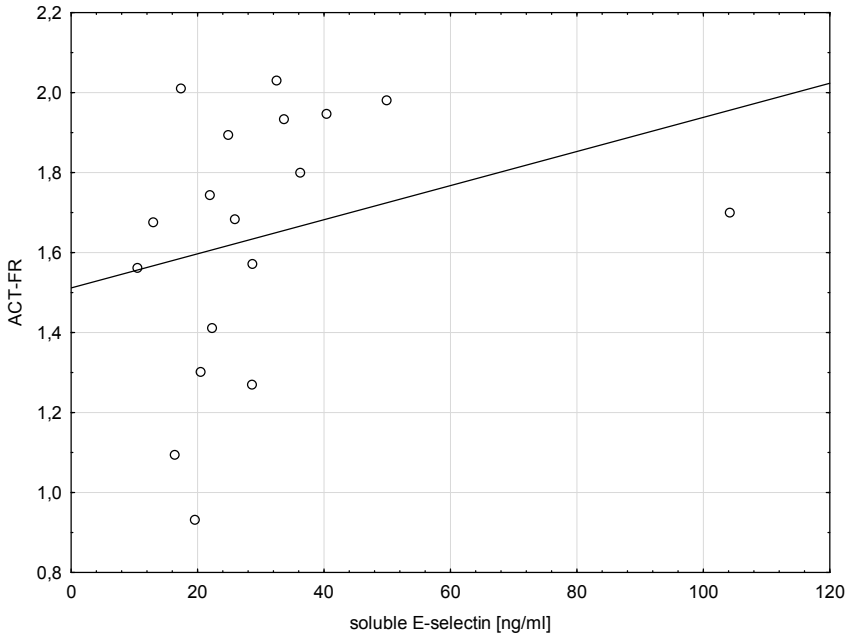

Figure 4. Correlation between α1-antichymotrypsin fucosylation ratio (ACT-FR) and soluble E-selectin in male patients with rheumatoid arthritis

Discussion

The function of raised levels of circulating selectins and increased fucosylation of glycoproteins in RA patients is obscure. Since sLe^x is known to be the simplest carbohydrate structure recognized by the selectins, glycoproteins that present sLe^x may function as the competitive inhibitors of interactions between selectins and their ligands during inflammatory reactions. Thus, the fucosylated proteins may represent a feedback mechanism, leading to the inhibition of leukocyte extravasation by blocking the selectins expressed on activated endothelium. On the other hand, the fucosylated proteins through binding with the soluble forms of selectins could inhibit their possible anti-inflammatory properties. Our hypothesis, that in vivo exists a common mechanism regulating fucosylation of proteins and serum selectin concentrations was promising. As far as we know, this is the first report comparing the fucosylation ratio of ACT with the concentrations of soluble forms of selectins in RA patients.

First of all, in the present study ACT-FR were significantly higher in men than in women, and only in male RA patients statistically significant correlations of ACT-FR with serum concentrations of sP- and sE-selectins were observed. These findings can be a result of altered fucosylation of glycoproteins due to hormonal factors [24]. Moreover, the considerably different results obtained for both sexes could be connected with the distinct RA phenotype between men and women [25, 26]. Our results are also concordant with previous report concerning fucosylation of α1-acid glycoprotein (AGP-FR) in RA [27]. In the study, Ryden et al. noted a weak correlation between AGP-FR only in men and concluded, that AGP-FR reflects rather basic inflammatory process than short-term variations of clinically assessed disease activity [27]. However, the limitation of our findings can be the fact that men accounted for the minority of the whole examined group. Therefore, the relevance and biological effect of these discoveries remains to be studied in a larger group of patients.

We also showed that ACT-FR significantly correlated with the concentration of RF and ACPA (especially in patients not treated with glucocorticoids), and was significantly lower in patients RF-negative vs. RF-positive. The last observation is concordant with the data reported by Ryden et al., who found a higher degree of AGP fucosylation in RF-positive women than in RF-negative women [27]. Moreover, our finding could be of a particular importance as it was previously reported that higher ACPA concentrations (particularly in RF-positive patients) are associated with increased disease activity in male patients with RA [28]. Thus, it is tempting to speculate that the level of ACT fucosylation not only indicates basal pro-inflammatory phenotype but can have some predictive value in disease outcome in RA.

Perspectives

To conclude, our findings demonstrated differences in fucosylation of ACT between male and female RA patients and revealed a relationship between ACT fucosylation and soluble selectins in men. Taken together, our results advocate towards better understanding of fucosylation mechanisms and their modulatory impact on immune system. It can become necessary to face future individualized therapeutic approaches of RA.

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Correspondence address:
Anna Olewicz-Gawlik, Department of Rheumatology and Clinical Immunology, Poznan University of Medical Sciences, Przybyszewskiego 39, 60–356 Poznan, Poland
tel. +48 (61) 8547210, fax +48 (61) 8547212, e-mail anolegaw@ump.edu.pl

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Acute kidney injury after cardiac surgical operations specially including coronary artery bypass graft operations

Adam Lipski[1], Marta Szymoniak-Lipska[1], Krzysztof Greberski[2]

[1] Students' Scientific Association of Cardiac Surgery, Department of Cardiac Surgery, Hospital J.Strusia, Poznan, Poland
[2] Department of Cardiovascular Prevention, The University of Medical Sciences Poznań, Department of Cardiac surgery Hospital J.Strusia, Poznan, Poland

ABSTRACT

Introduction. Acute kidney injury as abrupt loss of kidney function leads to accumulation nitric and non-nitric metabolites, toxins. It coexists with deep disorder of fluid balance. Most of cardiac surgical operations are performed using extracorporeal circulation (ECC). To the main risk factors of the postoperative dysfunction of kidneys belong: age above 70, congestive heart failure, previous CABG, preoperative creatinine concentration 124–177 μmol/L, diabetes type I, glucose concentration > 16,6 mmol/L, EEC longer than 3 hours and decreased cardiac output (CO).

Material and methods. The serum creatinine is not enough sensitive marker to diagnose early period of acute kidney injury because the serum creatinine increase occur later than true GFR changes and it needs time to accumulate. It depends on factors like: age, sex, weight, hydration status and what patient eat. NGAL (neutrophil-gelatinase associated lipocalin), cystatin C, KIM-1, IL-18, L-FABP are new markers of acute kidney injury which better than the serum creatinine concentration correspond with kidney injury. The risk factors of the acute kidney injury (AKI) are kidney hypoperfusion, microembolisation by bubbles or material particles and significant activation of humoral factors.

Results. One of the methods reducing mortality CSA-AKI (cardiac surgery associated kidney injury) is renal replacement therapy (RRT), which should be used in early period of acute kidney disease before severe symptoms and complications develop.

Conclusions. There is necessity to find early, easy and cheap markers of acute kidney injury which help decide if use renal replacement therapy. It increases the effectiveness of treatment and improves prognosis in this group of patients.

Keywords: Acute kidney injury (AKI), Cardiac surgery associated kidney injury (CSA-AKI), Renal replacement therapy (RRT).

Definition of acute kidney injury

Acute kidney injury as abrupt loss of kidney function leads to accumulation nitric and non-nitric metabolites, toxins. It coexists with deep disorder of fluid balance. Acute kidney injury (AKI) is defined (according to KIDGO 2012) [1] as increasing of creatinine concentration in serum 0,3 mg/dl (26,5 μmol/l) within 48 hours, or 1,5 times more of creatinine within 7 days, or diuresis < 0,5 ml/kg/h within 6 h. According to international RIFLE criteria (Risk, Injury, Failure, Loss and End-stage renal disease) (**Table 1**) we can evaluate risk, injury and development of kidney disease (loss of function and end-stage renal disease).

Table 1. Criteria RIFLE

Class	GFR Criteria	Urine Output Criteria
Risk	Increased Serum Creatinine x 1.5 or GFR decrease >25%	< 0.5 ml/kgmc/h within 6 h
Injury	Increased Serum Creatinine x 2 or GFR decrease > 50%	< 0.5 ml/kgmc/h within 12 h
Failure	Increased Serum Creatinine x 3 or GFR decrease > 75% or Serum Creatinine ⩾ 4mg/dl with acute rise ⩾ 0.5 mg/dl	< 0.3 ml/kgmc/h within 24 h or anuria within 12 h
Loss of function	Persistent acute renal failure = complete loss of kidney function >4 weeks	
End-stage renal disease (**E**SRD)	End stage kidney disease (> 3 months)	

Risk factors of acute kidney insufficiency

Among the most often performed cardiac surgical operations we can mention Coronary Artery Bypass Graft (CABG) with or without off-pump coronary artery bypass (OPCAB), surgery of the mitral, aortic and tricuspid valve, surgery of aneurysm of the ascending aorta, aneurysm of the arch, surgery of aorta dissection. Apart from OPCAB most of these procedures are performed using extracorporeal circulation (ECC). To the main risk factors of the postoperative dysfunction of kidneys belong: age above 70, congestive heart failure, previous CABG, preoperative creatinine concentration 124–177 µmol/L, diabetes type I, glucose concentration > 16,6 mmol/L, EEC longer than 3 hours and decreased cardiac output (CO) (**Table 2**).

Etiology of acute kidney injury after cardiac surgical operations

Prevalence of acute kidney injury after cardiac surgical operations are between 7,7% [2] and 42% [3]. One of the main acute kidney injury (AKI) risk factors is kidney hypoperfusion connected with low blood volume and

Table 2. Risk factors of postoperative AKI for patients who were underwent CABG

RIsk factors	Relative risk (95% CI-Cardiac index)
preoperative	
age	
70–79	1.6 (1.1–2.3)
80–95	3.5 (1.9–6.3)
congestive heart failure	1.8 (1.3–2.6)
Previous CABG	1.8 (1.2–2.7)
Preoperative creatine concentratiom 124–177 µmol/L	2.3 (1.6–3.4)
Diabetes	
Diabetes type I	1.8 (1.1–3.0)
Glucose concentration > 16.6 mmol/L	3.7 (1.7–7.8)
Midoperetive and postoperetive	
ECC longer than 3 hours	2.8 (1.9–7.2)
Decreased cardiac output*	
significantly	4.5 (2.9–7.2)
moderately	3.1 (1.9–4.9)
mildly	4.3 (2.2–8.5)

**Significantly: Intra-aortic balloon pump (IABP); moderately : 1) cardiac index < 1.5 L/min/m^2 surface of the body during at least 30 minutes or at least three inotropes, 2) congestive heart failure confirmed by CI < 1.5 L/min/m^2, 3) PCWP – pulmonary capillary wedge pressure > 18 mmHg, or 4) central venous pressure > 12 mmHg; mildly: congestive heart failure confirmed by rales, murmurs, chest radiograph, or widening of the jugular veins.*

disorder of blood pressure. If there were not appropriate procedure the condition of GFR functional decreasing lead to actin cytoskeleton disintegration, loss intercellular tight junctions, disorder of integrins functions and in result epithelium of convoluted tubule desquamation [4–6]. Another reason of acute kidney injury after cardiac surgical operations is microembolisation which is caused bubbles or material particles [7]. During cardiac surgical operation blood is undergone shear strain when it has contact with artificial material in extracorporeal circulation. It leads to red blood cells damage and hemoglobin and iron release that have redox activity. Redox reactions induce lipid peroxidation and tissue damage [8]. After cardio surgical operation with using cardiopulmonary bypass there is significant activation of humoral factors. Artificial surface activates XII coagulation factor and stimulates coagulation cascade and in result also fibrinolysis. Complement system is activated in classical pathway and alternative pathway. In the same time there are platelet activation, degranulation, adhesion to endothelium, neutrophil and endothelium activation. They result in increasing concentration of adhesion molecule, cytokines, chemokines, lytic enzymes and reactive oxygen species [9–16].

Markers of acute kidney injury

The serum creatinine is not enough sensitive marker to diagnose early period of acute kidney injury because the serum creatinine increase occur later than true GFR changes and it needs time to accumulate. The serum creatinine concentration depends on factors which is not directly connected with kidney like: age, sex, weight, hydration status and what patient eat.

NGAL (neutrophil-gelatinase associated lipocalin) is protein secreted into urine by thick ascending limb of loop of Henle and collecting duct cells. NGAL takes part in iron complex chelation and recirculation of iron complex by endocytosis that protects renal tubule from ischemic [17]. Excretion NGAL with urine significantly increases in AKI caused ischemia [18], especially after cardiac surgical operations [19] or kidney transplant [20, 21], after application big dose contrast agents [22] or other nephrotoxic substance [23, 24]. There was not observed significant increase of NGAL excretion after coronary catheterization [25].

Cystatin C in serum is earlier than creatinin marker of kidneys' function used as well to identify progressive changes as to detect dysfunction. Cystatin C is non-glycosylated protein, endogenic inhibitor of cysteine proteases, produced and released into blood in constant rate by all nucleated cells. The concentration of cystatin C in serum is independent of age, sex, race, body weight and hydration status and can be measured using easy nephelometric method. Relatively high concentration in body fluid, low mass (13,3 kDa) and positive electric charge of this protein ensure easy glomerular filtration to primary urine. In proximal coils it's completely reabsorbed and catabolized. Because of constant rate of production the value of concentration cystatin C can be a marker of glomerular filtration what is more it does not depend on any factors as: infection, liver diseases, inflammatory diseases [26, 27]. Using cystatin C dysfunction of kidney's can be detected in critical state patients with AKI (24–48 hours earlier than using creatinin). However it is not a specific marker for AKI, because it is rather an early marker of glomerular filtration than a marker of dysunction of coils [28–30].

KIM-1 (kidney injury molecule 1) – it is a transmembrane orphan receptor produced produced in a large amount in proximal coil because of toxic or ischemic injury. The extracellular domain is removed in urine. vi the increase its excretion is combined with surgery of abdominal aorta aneurysm, heart (precedes the increase of creatinine), but it was not detected in contrast nephropathy [31, 32].

IL-18 is proinflammatory cytokin released into urine by epithelium of proximal ducts after some kind nephrotoxic factor occurred. Concentration above 100 pg/mg creatinin corresponds well with risk of AKI and death in patients with acute respiratory disorders syndrome [33] and it predicts that the kidney will work with deley after trasplantation [34]. Increase in concentration of Il-18 can be detected in first hours after cardiovascular surgery in children [35]. Examination of IL-18 in urine allows to discern very early stage of kidney injury caused by ischemia or coil nephrotoxin and let us excluded prerenal azotemia, chronic kidney disease and urinary tract infection [36]. Furthermore IL-18 is a predictor of AKI severity and probability of death.

L-FABP is cytoplasmic protein responsible for intracellular transport of long-chain fatty acid. There are known 9 types of proteins transporting fatty acid and L-FABP is one of them. They are relatively specific to the tissue. L-FABP can be found in hepatocytes, enterocytes, and the proximal coil cells. In different kidneys injuries concentration L-FABP in urine increases. It was proved that L-FABP is a adequate marker of AKI after cardiac surgery. Its concentration increased in urine 94 times and 45 times more than in people who were not

underwent such procedures properly in 4. and 12. hour after surgery. The concentration of L-FABP increases faster than of creatinin [37]. It was revealed that the concentration of L-FABP is useful in in the monitoring of contrast-induced nephropathy [38], cis-platin induced nephropathy [39], and also in the staging of chronic kidney disease [40].

Methods of reduction mortality connected with acute kidney injury (AKI)

Prevalence of acute kidney injury connected with cardiac surgical operations (cardiac surgery associated kidney injury – CSA-AKI) in early after-operation period may be even 30% and it increases because more people are operated in old age and with co-exited disease e.g. diabetes or chronic kidney disease [41] One of the methods reducing mortality CSA-AKI is renal replacement therapy (RRT), which should be used in early period of acute kidney disease before severe symptoms and complications develop. Increase serum creatinine concentration and GFR decrease occur in 2^{nd}–3^{rd} day after damaging agent. It provides big loss of nephrons which often is irreversible. In many department of cardiac surgery the serum creatinine serum concentration is still main way to evaluate kidney function. There is necessity to find early, easy and cheap markers of acute kidney injury which help decide if use renal replacement therapy. It increases the effectiveness of treatment and improves prognosis in this group of patients.

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Correspondence address:
Adam Lipski
1/41 Wawrzyniaka Str.
60-506 Poznań, Poland
phone: +48 791245258
e-mail: adamadam.l@wp.pl

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Characteristic of no-synthase of peripheral blood lymphocytes of patients with rheumatic pathology

Ulyana Efremova, Nataliya Lychkovska, Roman Fafula, Zinoviy Vorobets

Danylo Halytsky Lviv National Medical University, Ukraine

ABSTRACT

It is known that NO is a ubiquitous mediator which acts as a universal modulator of various functions in organism and is produced by three isoforms of NO synthase. Nowadays the role of NO in the development of autoimmune diseases is actively studied. However, it remains unclear the biochemical and biophysical mechanisms of disturbances of NOS activity in blood lymphocytes at autoimmune process. The aim of present work is to study the kinetic properties of NO-synthase of peripheral blood lymphocytes of patients with rheumatic pathology. The study was carried out on peripheral blood lymphocytes isolated from patients with rheumatoid arthritis and ankylosing spondylitis. NOS activity was determined on the saponin-permeabilized blood lymphocytes. The difference between the values of NADPH oxidation with L-Arg and with inhibitor L-NAME reflects the value of the NADPH oxidation, ie total NOS activity. The kinetic properties of NO-synthase in peripheral blood lymphocytes of patients with rheumatic pathology were studied. It was found that the development of rheumatic pathology is associated with an imbalance in the NO synthesis and changes of kinetic parameters of NOS. It was shown that reduction in eNOS activity is accompanied by a sharp increase in activity of its inducible form. It was established that inhibition of eNOS occurs by noncompetitive type. NO production in lymphocytes of patients with rheumatic diseases is mainly realized by iNOS, whereas under normal physiological conditions endothelial form of the enzyme is being involved.

Keywords: NO-synthase, nitric oxide, lymphocytes, rheumatoid arthritis, ankylosing spondylitis.

Introduction

At the end of the 20th century it has been found that in any living organism a nitric oxide (NO) is produced in large concentrations. Further study of its biological role revealed that NO is a gaseous messenger which acts as a universal modulator of various functions in organism [1, 2]. NO is a ubiquitous mediator that is produced by three isoforms of NO synthase (NOS): neuronal (nNOS I), endothelial (eNOS II) and inducible (iNOS III) [3]. Two isoforms of NO-synthase are classified as constitutive NO synthase – neuronal (nNOS I) and endothelial (eNOS II). Their designations indicate the cell types in which these isoforms have been identified. Functioning of constitutive forms is regulated by Ca^{2+} ions. eNOS is a Ca^{2+}-dependent and after any stimulant which causes an increase in the intracellular Ca^{2+} concentration it synthesizes very small amounts of NO which has biomodulative effects. Also eNOS plays a key role in ensuring the constant "basal" NO level.

The third isoform of NO-synthase is inducible (iNOS II), as its activation induced by cytokines, endo- or exotoxins. Its synthesis occurs over 6–8 hours after their actions. iNOS is Ca^{2+}-independent isoenzymes. In physiological conditions it is inactive. NO which is produced by the activation of inducible NOS is primarily designed for the protection of organism [4]. This isoform is associated with immunological and infectious stimuli and is expressed in macrophages, neutrophils and endothelial cells. The NO production by activated macrophag-

es confirms a cytotoxic and cytostatic role of NO in immune system. It should thus be noted that NO can also be produced by non-enzyme interaction of arginine and hydrogen peroxide [5]. Also it has been found that eNOS produces low concentrations of NO, whereas iNOS synthesizes high concentrations of NO [4].

Although isoenzymes are products of different genes and they have different functions, the differentiation on inducible and constitutive NO synthesis is conditional, since they form a single product – a NO molecule, which diffuses easily through cell membranes and does not require receptors to perform its effects. The balance between physiological, regulatory and/or cytotoxic properties are largely due to the local concentration of NO and oxidative status of the tissue in which NO is synthesized and realizes its effects [6, 7].

Nowadays the role of NO in the development of autoimmune diseases is actively studied [8, 9]. It has been proved a key role of NO in the regulation of immune responses and its participation in almost every stage of inflammation. Inside the cells NO activates some enzymes and inhibits the others, thus participating in the regulation of cellular functions. High NOS activity leads to NO accumulation and initiation of pathological processes in the cell [10, 11]. Herewith the inhibition of lymphocytes proliferation and increase of apoptosis of lymphocytes and macrophages occurs which leads to secondary immunodeficiency [12]. The NOS activity and NO level along with other parameters may indicate the state of the cell system and be auxiliary prognostic indicators for various diseases, including rheumatic.

Nowadays, a rheumatic diseases are considered as one of the most common pathologies of modern society. They are among the most difficult diseases by their severity and selection of adequate treatment. Most chronic inflammatory rheumatic diseases are mediated in a particular stage by immune disorders. The reason of immunocomplex destruction in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (ASA) is the disturbances in the regulation of the immune response. This is due to imbalance in function of T-and B-lymphocytes, which play an important role in compensatory-adaptive reactions of organism.

There is a large number of studies devoted to enzymatic spectrum of blood lymphocytes under various diseases, but the studies of NOS activity of blood lymphocytes in patients with RA and AS are limited.

As a result of our previous studies on the enzymatic activity of NOS in peripheral blood lymphocytes it was shown [13] that NOS activity in patients with RA and ASA is significantly different from that in healthy persons. After the treatment the NOS activity in patients approaches to its control values. However, it remains unclear the biochemical and biophysical mechanisms of disturbances of NOS activity in blood lymphocytes at autoimmune process. The aim of this work was to study the kinetic parameters of NOS of peripheral blood lymphocytes of patients with RA and ASA.

Materials and methods

The study was carried out on peripheral blood lymphocytes isolated from patients with RA and ASA, treated in Lviv Regional Clinical Hospital (Ukraine). All patients were divided into two groups: patients with RA (n = 40) and patients with ASA (*n* = 30). Control group were practically (clinically) healthy persons representative by age and sex (*n* = 30). All patients and donors gave written informed consent to participate in research (Ethical Committee Approval Protocol No 8 from October 22, 2012).

Isolation of lymphocytes

Mononuclear peripheral blood lymphocytes were isolated from heparinized freshly obtained blood by ficoll-triombrast with gradient density ρ = 1.08 g/cm^3 [14]. Integrity and viability of blood lymphocytes evaluated using trypan blue staining in all experiments was at least 95 % [15].

NOS activity was determined on the saponin-permeabilized blood lymphocytes. For permeabilization of lymphocytes membrane and disclosure of enzymatic activity lymphocytes were incubated for 10 min with moderate shaking in a solution containing saponin in a concentration of 0.2% [16, 17]. Protein content in lymphocytes mixture was determined by Lowry method [18].

Determination of NOS

Determination of NOS activity in permeabilized lymphocytes was carried out in the reaction mixture (at 37°C) containing 80 mM Tris-HCl buffer (pH 7.4), 5 mM $CaCl_2$, 0.15 mM *L*-Arg, 0.12 mM NADPH. The reaction was initiated by the introduction of an aliquot of blood lymphocytes (70 µl) in the reaction mixture. Protein content in the sample did not exceed 50–75 µg. The difference between the values of NADPH oxidation with *L*-Arg and with inhibitor *L*-NAME reflects the value of the NADPH oxidation, ie total NOS activity. The test samples were estimated spectrofotometrically against control samples at 340 nm and then incubat-

ed for 20 min at 37 °C. The reaction was stopped by the introduction of 0.05 ml of 1.5 M $HClO_4$ and then decrease in extinction was registered [19]. NOS activity was expressed in nmol NADPH oxidase per 1 min per 1 mg of lymphocytes protein [20].

The activity of Ca^{2+}-independent iNOS was determined similarly, adding to the incubation medium Ca^{2+} chelators EGTA (4 mM) instead of $CaCl_2$. The activity of Ca^{2+}-dependent NOS isoforms was calculated as the difference between total NOS activity and Ca^{2+}-independent NOS activity.

Kinetic analysis

Kinetic analysis of the enzyme reaction was performed in a standard incubation system (as described above) with modified physical and chemical characteristics or the respective components (the substrate concentration, incubation time, protein content and detergent concentration). The kinetic parameters characterizing the NO-synthase reaction – the initial (instantaneous) reaction rate (V_0), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time half saturation) τ were determined according to [21]. The apparent affinity constant for *L*-arginine ($K_{L\text{-}Arg}$) and maximum reaction rate (V_{max}) were determined by Lineweaver-Burk plot [22]. Kinetic and statistical calculations were carried out using the software MS Office computer programs. The results were treated by methods of variation statistics using Student *t*-test. The equation of the straight line that approximates the experimental data the best was calculated by method of least squares. The absolute value of the correlation coefficient *r* was from 0.85 to 0.95. The significance of the calculated parameters of line was tested by the Fisher's *F*-test. The accurate approximation was when $P \leqslant 0.05$.

Results

Kinetic analysis of NOS activity on concentration of L-arginine

Isoenzymes of NOS are dioxygenases which use molecular oxygen and NADPH for the transformation of *L*-arginine to *L*-citrulline and NO. Arginine is the basic amino acid substrate for NO production by all NOS isoforms.

For this reason, changes in the *L*-arginine concentration in the incubation medium affect the rate of NO-synthase reaction. The dependence of the NO-synthase activity on substrate concentration in the incubation medium is determined by the apparent affinity constant to the substrate K_{LArg}. For its determination the *L*-arginine was added to the incubation medium in concentrations ranging from 0.1 to 30 mM (at constant concentration of $CaCl_2$ – 10 mM and NADPH – 0.12 mM). It was observed a monotonic increase in the enzymatic activity of two isoforms of NOS reaching a plateau (**Figure 1**). As can be seen from figure 1 the eNOS activity in patients with RA is reduced in comparison with the value in healthy donors in the whole range of *L*-arginine concentrations. Reduction of eNOS activity is accompanied by a sharp increase in activity of inducible form. The optimum substrate concentration for both enzymes is within 20–30 μM. The dependence NOS activity on *L*-arginine concentration for patients with ASA has an identical character.

It was determined the main kinetic parameters of *L*-arginine hydrolysis to elucidate the possible mechanism of change in NOS activity in patients with RA and ASA (**Figure 2**).

Linearization of concentration curves in Lineweaver-Burk plot for iNOS has an identical character. The main kinetic parameters NOS of blood lymphocyte of donors and patients with RA and ASA were calculated by linearization of the data in Lineweaver-Burk plot (**Table 1**).

Data in table 1 show that the maximum rate of hydrolysis of *L*-arginine for eNOS of blood lympho-

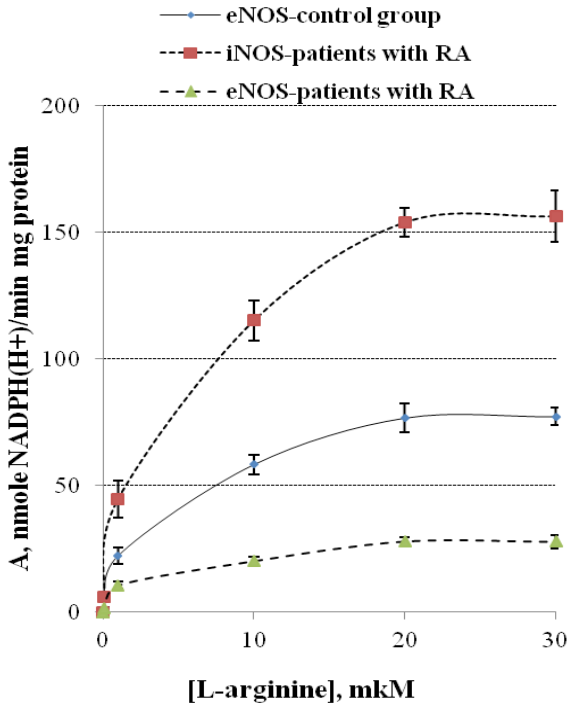

Figure 1. Concentration dependence of L-arginine effect on NOS activity of saponin-permeabilized peripheral blood lymphocytes of patients with rheumatoid arthritis, M ± m, n = 6–8

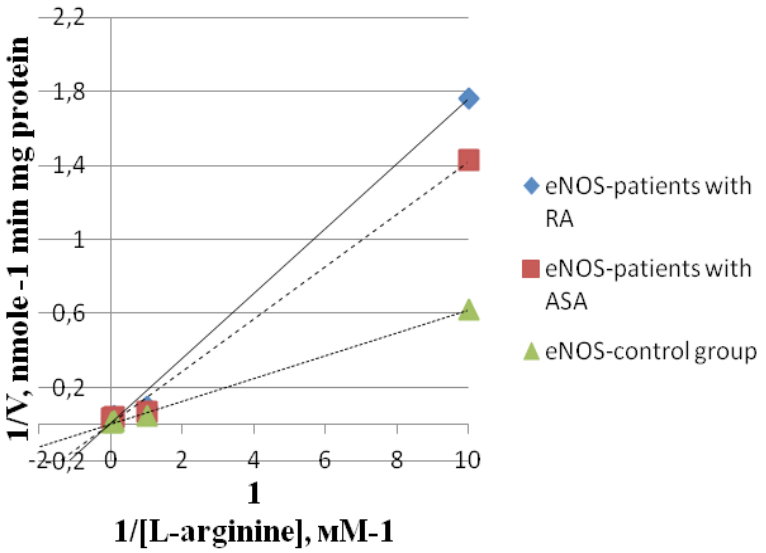

Figure 2. Linearization of concentration curves in Lineweaver-Burk plot, here V is eNOS activity of saponin--permeabilized peripheral blood lymphocytes of patients with rheumatoid arthritis and ankylosing spondylitis, n = 4-6; r > 0.85; F < 0.005

Table 1. Kinetic parameters of NOS of blood lymphocytes of patients with RA and ASA, defined by L-arginine, M ± m, n = 6–8

Kinetic parameters	Patients with RA	Patients with ASA	Control group
		eNOS	
V_{max}, nmol NADPH(H^+)/min· mg protein	92.2 ± 12.8 ***	108.8 ± 8.2 ***	235.6 ± 44.0
$K_{L\text{-}Arg}$, μM	16.3 ± 0.4	15.8 ± 1.5	14.6 ± 2.7
		iNOS	
V_{max}, nmol NADPH(H^+)/min· mg protein	164.3 ± 10.2	142.4 ± 11.0	–
$K_{L\text{-}Arg}$, μM	2.8 ± 0.5	2.6 ± 0.4	–

**** P < 0.001 compared to healthy donors*

cytes of healthy persons is 2.5 and 2.2 times greater than this value for eNOS of patients with RA and ASA accordingly. However, affinity constants for *L*-arginine in all studied groups were not significantly different. Hence we can conclude that affinity of eNOS for *L*-arginine does not change in patients with RA and ASA. Thus, in patients with rheumatic pathology the inhibition of eNOS in immunocompetent cells occurs by non-competitive type, by reducing the speed of the enzyme (value of V_{max} decreases).

The maximum rate of *L*-arginine hydrolysis for iNOS (which is not identified in normal and is significantly activated in rheumatic diseases) differs from this value for eNOS of blood lymphocytes in healthy persons (in 1.4 times greater than in patients with RA and 1.6 times greater than in patients with ASA) Affinity constant for *L*-arginine for iNOS of blood lymphocytes in patients with rheumatic disorders is lower than for eNOS in healthy persons (in 5.2 times in patients with RA and in 5.6 times in patients with ASA).

Kinetic analysis of NO-synthase reaction on time

For studying the peculiarities and the mechanism of NOS functioning the initial (instantaneous) reaction rate (V_0), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time half saturation) (τ) were determined [21]. For determination of these kinetic parameters of NOS the dynamics of NADPH(H^+) accumulation, indicating NO synthesis, was examined. Suspension of lymphocytes was incubated in the standard incubation medium for various periods of time (0–30 min.). These experiments show that kinetics of NO-synthase reaction by saponin-permeabilized lymphocytes is reflected by curves that tend to saturation (**Figure 3**).

Analysis of the results shows that kinetics of NO production by eNOS is consistent with the first-order reaction in the range 0–20 min. In this time interval the dependence of NO production on the incubation period is almost linear. Therefore, in further experiments the incubation time of lymphocytes and, therefore, NO-synthase reaction is 20 min.

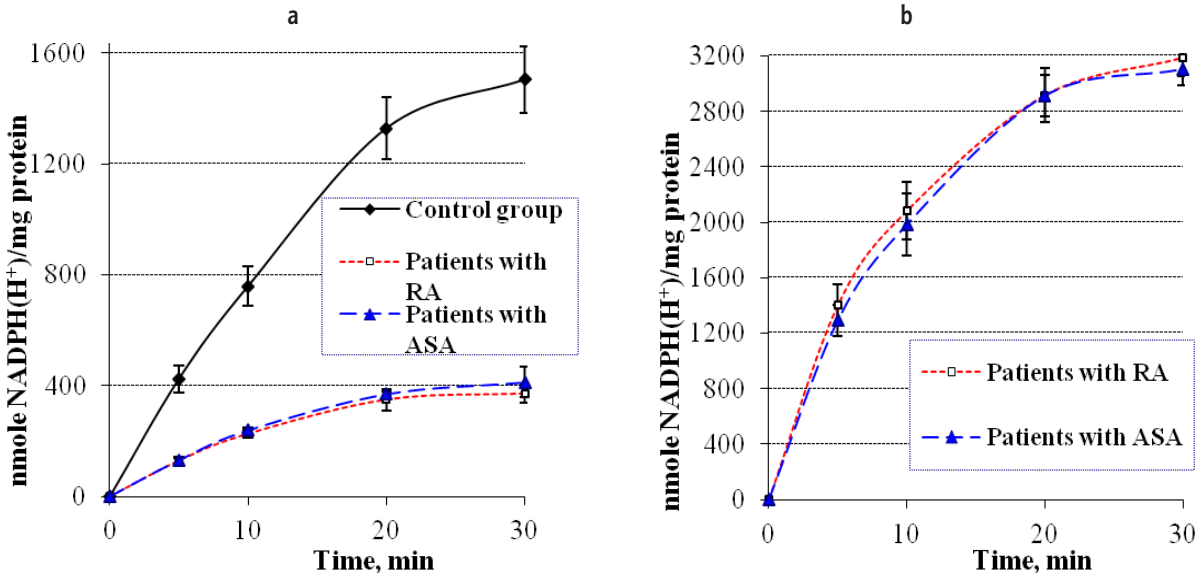

Figure 3. Dynamics of NADPH(H+) production in NOS reaction of blood lymphocytes by eNOS (a) and by iNOS (b) in patients with RA and ASA, M ± m, n = 6–8

As can be seen from **Figure 3a** in the whole range of time, the value of NO produced by eNOS of blood lymphocytes of patients with rheumatic diseases is much lower compared to value in healthy donors. However, the value of NO produced by iNOS (**Figure 3b**) of blood lymphocytes of patients with rheumatic diseases is significantly higher than these values in donors.

By linearization of the data in the coordinates *P/t* on *P* the main kinetic characteristics of eNOS and iNOS of blood lymphocytes in patients with RA were calculated (**Table 2**, **Figure 4**). In patients with ASA the linearization curves of NADPH accumulation by endothelial and inducible form of NOS have a similar appearance.

As can be seen from Table 2 the values of the kinetic parameters of NO production by eNOS of blood lymphocytes of patients with rheumatic diseases and healthy persons differ significantly. The maximum instantaneous rate of eNOS is greater in 3 times in healthy donors compared with values in patients with RA and ASA. Maximum amount of reaction product by eNOS in control group exceeds this value in patients with RA and ASA in 5.1 and 4.2 times accordingly.

The results of kinetic analysis indicate that NO production by iNOS is much more intense than by eNOS, and, in turn, NO production by eNOS in the control group is quicker and more active than in patients with rheumatic pathologies. The maximum instantaneous rate of iNOS reaction exceeds this value of eNOS reaction in patients with RA and ASA in 12 and 10.8 times accordingly. Maximum amount of reaction product by iNOS exceeds this value for eNOS in patients with RA and ASA in 6.5 and 5.7 times respectively.

Kinetic analysis of NOS activity on concentration of protein

Taking into account that enzyme activity depends on the protein content in incubation medium, the NOS

Table 2. Kinetic parameters of L-arginine hydrolysis by NOS of blood lymphocytes of patients with RA and ASA, M ± m, n = 6–8

Kinetic parameters	Patients with RA	Patients with ASA	Control group
		eNOS	
V_0, nmol NADPH(H^+)/min· mg protein	33.9 ± 2.4***	32.8 ±2.7***	98.0 ± 7.2
P_{max}, nmol NADPH(H^+)/mg protein	690.1 ± 83.0***	827.6 ± 91.0***	3512 ± 306
τ, min	21.4 ± 5.9 **	26.0 ± 6.8****	36.6 ± 4.6
		iNOS	
V_0, nmol NADPH(H^+)/min· mg protein	405.2 ± 50.3	356.0 ± 37.4	–
P_{max}, nmol NADPH(H^+)/mg protein	4524.2 ± 282.6	4725.9 ± 302.8	–
τ, min	11.6 ± 1.9	13.7 ± 2.1	–

*** P < 0.01*
**** P < 0.001 compared to healthy donors*

reaction was initiated by inclusion of protein with concentrations ranging from 25 to 150 μg/ml in lymphocyte mixture (**Figure 5**). It was investigated that a gradual increase in lymphocyte protein concentration in the incubation medium leads to an increase in V_0 of NOS reaction.

The dependence of the NADPH(H^+) production on the protein content in incubation medium has the same character both for eNOS and iNOS. However, the data indicate that maximum instantaneous rate of NADPH(H^+) production by eNOS in patients with RA and ASA is significantly different from that of healthy donors (**Table 3**).

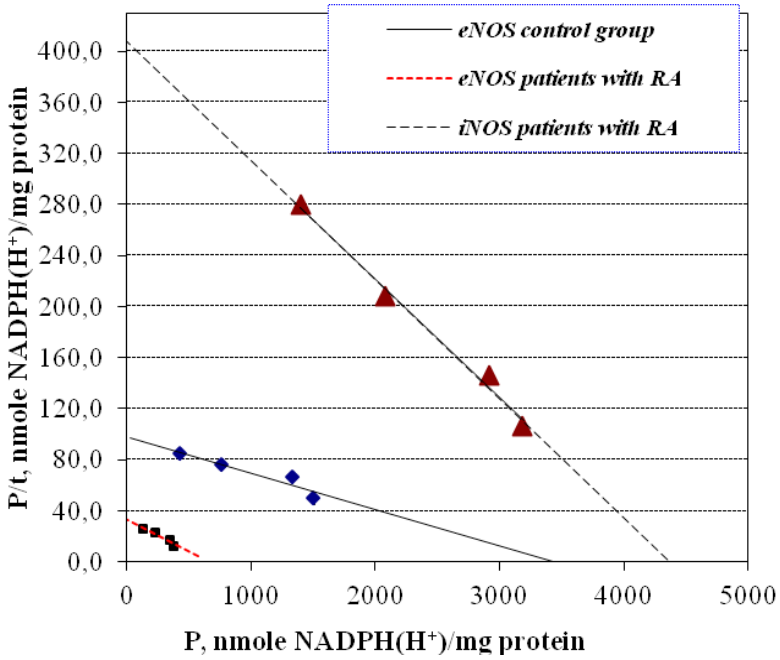

Figure 4. Linearization of curves of NADPH(H+) accumulation by eNOS and by iNOS of saponin-permeabilized peripheral blood lymphocytes of patients with rheumatoid arthritis in coordinates [P/t; P], n = 6–8; r > 0.9; F < 0.02

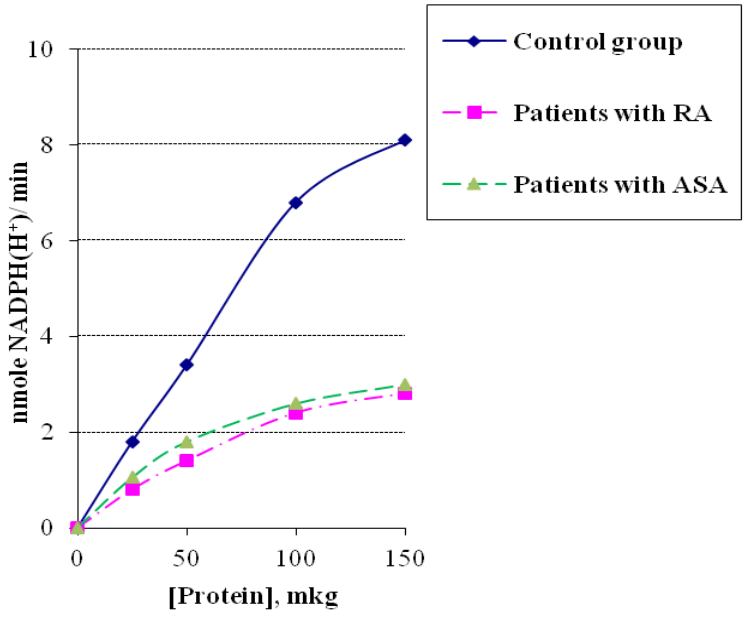

Figure 5. Dependence of the initial rate of eNOS reaction of saponin-permeabilized blood lymphocytes of patients with RA and ASA on protein content, M ± m, n = 4–6

Table 3. The kinetic parameters of NADPH(H^+) production by eNOS of saponin-permeabilized blood lymphocytes of patients with RA and ASA, M ± m, n = 4–6

Kinetic parameters	Control group	Patients with	
		RA	ASA
V_0, nmol NADPH(H^+)/min· mg protein	53.6 ± 1.0	18.6 ± 0.5***	17.6 ± 1.4***

**** P < 0.001 compared to healthy donors*

Analysis of the dependence of NOS activity on the detergent (saponin) concentration

Studying the NOS activity the different methodological approaches (studies on isolated subcellular structures, on whole cells or on cell homogenates) are used. Study of the NOS properties on isolated membrane fractions or lymphocyte mitochondria is difficult due to their gradual inactivation during preparative obtain of fractions of subcellular structures.

The aim was to pick up the optimal conditions for NOS determination using saponin as a detergent. This substance is capable of binding to membrane proteins by hydrophobic bonds, while interacting by polar groups with water. This allows molecules of detergent to loosen the membrane without disrupting its structure and function [31]. Saponin was used in the concentration range from 0.02 to 0.3%.

As can be seen from **Figure 6** in a wide range of saponin concentrations (0.02–0.3%) curves of enzyme activity on the detergent concentration are dome shaped. The maximum of NOS activities is observed for saponin concentrations of 0.1–0.2%. This range of saponin concentrations can be considered as the most appropriate for practical use in experiments to study the kinetic and catalytic properties of NOS. Lower saponin concentrations (0.02–0.04%) do not disclosure of NOS activity fully. Dependences NOS activity on saponin concentration for lymphocytes of patients with ASA have a similar appearance.

Discussion

The availability of *L*-arginine is a potential mechanism for controlling the NO production, since most cell types can not synthesize arginine and require arginine intake from outside [23, 24, 25].

Physiological needs of most tissues and organs of mammals in arginine are satisfied with its endogenous synthesis and/or intake of food. But this amino acid is essential for young individuals and adults under stress or illness. Arginine is an essential precursor for the synthesis of proteins and many biologically important molecules, such as ornithine, proline, polyamines, creatine and agmatine. However, the main role of arginine in organism is to be a substrate for NO synthesis [26].

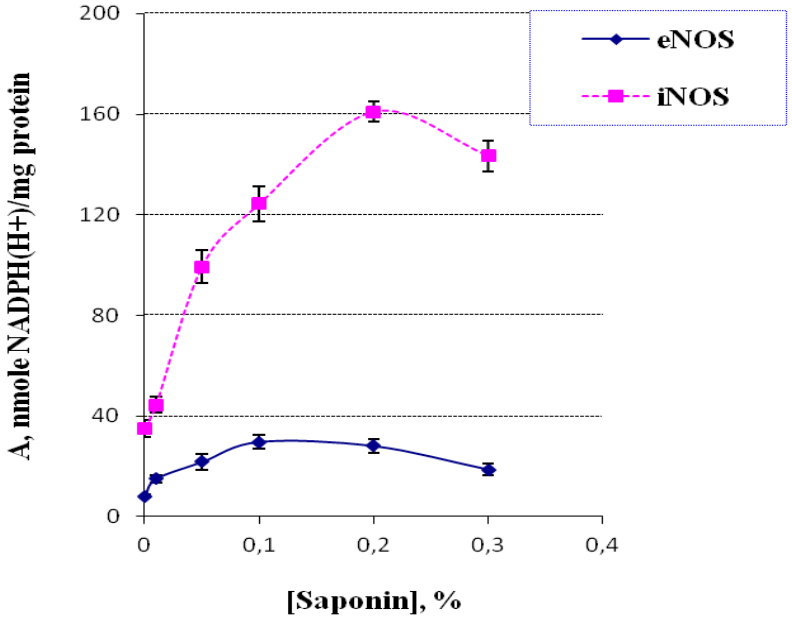

Figure 6. The dependence of eNOS and iNOS activity of saponin-permeabilized blood lymphocytes of patients with RA on saponin concentration, M ± m, n = 4

However, local bioavailability of *L*-arginine for NOS may be reduced due to the increased activity of *L*-arginase, which competes with NOS for the same substrate. Arginases metabolize arginine to ornithine and urea [23, 27, 28].

If iNOS is induced by classically inflammatory cytokines (IL-1, TNF-α, γ and IFN-γ and IL-2), the humoral proinflammatory cytokine (IL-4, IL-10, IL-13 and TGR-β) induce the expression of arginase. Endotoxin induces both iNOS and arginase I [27]. iNOS provides a regulating effect on arginase activity through production of hydroxy-*L*-arginine which is an intermediate product in the NO production. Arginase, in turn, can regulate NO synthesis through depletion of arginine availability [28].

Taking into account that cellular NO production in immune cells is completely dependent on the presence of *L*-arginine [29], a sharp increase in iNOS activity in blood lymphocytes of patients with RA and ASA is probably associated with an increase in substrate concentration.

Based on our data, we assume that in white blood cells of patients with RA and ASA the NO production by iNOS is much more intense than by eNOS. NO production by eNOS in patients with rheumatic pathology is slower and less active than in healthy donors. Thus, we can conclude that in lymphocytes of patients with RA and ASA the NO production is realized by inducible isoform of NOS mainly, and under normal physiological conditions involving endothelial enzyme form.

Using whole cells the different NOS isoforms are in latent state and inaccessible to substrates. Therefore, testing their activities possible after prior disturbance of integrality of lymphocytes plasma membranes. This can be achieved by introducing a substance leading to perforation of plasma membranes (detergent) in the incubation medium. Using a suspension cells pretreated with detergent is adequate for correct testing of NOS of subcellular structures. Under these conditions the natural interrelation of intracellular stores is obey [30].

Similar studies of dependence of enzyme activity on the saponin concentration were carried out by other researchers. It was shown that similar saponin concentrations are used to uncover latent activity of bot h membrane and cytosolic enzymes [31]. Using such methodological approach for testing the enzymes is also confirmed by ultrastructural study [32].

Under conditions of rheumatic pathologies the effects of NO are different. On the one hand, a disturbance of constitutive NO production is associated with a decrease in the eNOS activity. On the other hand, these conditions can lead to iNOS hyperactivation and excess production of cytotoxic amounts of NO.

Conclusions

It was shown that reduction in eNOS activity is accompanied by a sharp increase in activity of its inducible form. It was established that inhibition of eNOS occurs by noncompetitive type. NO production in lymphocytes of patients with rheumatic diseases is mainly realized by iNOS, whereas under normal physiological conditions endothelial form of the enzyme is being involved.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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Correspondence address:
Ulyana Efremova
Department of Medical Biology
Danylo Halytsky Lviv National Medical University
69 Pekarska Str., 79010 Lviv, Ukraine
phone: + 38 0930390437
fax: +38 0322757632
e-mail: efremova_ulyana@ukr.net

ORIGINAL PAPER

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Selected aspects of end-of-life care in the Intensive Therapy Unit

Sylwia Miętkiewicz, Bożena Siczyńska, Danuta Dyk

Department of Anaesthesiology and Intense Care, Faculty of Life Sciences, Poznan University of Medical Sciences, Poland

ABSTRACT

End-of-life (EOL) care represents a significant component of palliative/hospice care. It is applied in terminal hours, days or weeks of life, which require a very scrupulous, professional protection and paliative treatment, targeted at alleviation of sufferings. Mortality in wards of anaesthesiology and intense therapy (ICU) reaches 17%-24% and frequently, despite application of modern therapeutic methods, the patient dies.

This paper aims at drawing readers' attention to selected aspects of care given to patients and their families in the end-of-life in ICU. Dying represents a very difficult process not only for the patient and his/her family but also for the staff taking care over the patient. It represents a dynamic process and, therefore, the management should be modified depending on altering condition of a patient and his/her relatives; it is linked to adaptation of care and symptomatic treatment and to meeting half-way anxieties of patient's relatives. The patient should be viewed in his/her entire set of biological, psychic, spiritual and social needs and it should be born in mind that securing an appropriate comfort of terminal moments in life is occasionally more important than a heroic battle for every minute of life. In the terminal period of life quality of life depends on an appropriate communication, acceptance that the patient and his/her family keep the key for undertaking decisions, for a continuous care, emotional support, for assurance of an appropriate care, correct symptomatic treatment, spiritual, emotional and organizational support.

Keywords: end-of-life, care in terminal period of life, communication, LPC, DNAR.

Introduction

End-of-life (EOL) patient care and preservation of human dignity in a disease and death is particularly valid in wards of anaesthesiology and intense therapy (ICU). Mortality in such wards reaches 17%-24% [1–3]. The care in ICU frequently oscillates around palliative care. The palliative/hospice care (PHC) represent unequivocal terms. World Health Organization (WHO) accepted the following definition of palliative care: „Palliative care denotes such a care over ill patients and their close and dear which improves to maximum their quality of life by anticipation, prevention and treatment of suffering. Throughout the entire disease palliative care involves satisfying physical, intellectual, social and spiritual needs and support of independence of the patient, securing access to information and satisfaction of patient's choices" [4, 5]. End-of-life care is not identical to PHC but it represents its significant component; it finds application in care of a patient who is close to death (i.e. in terminal days or weeks of life), but who remains still alive and who requires a very careful, professional care and palliative treatment, targeted at providing relief from suffering [4]. It happens that a patient is admitted to the ward in a situation when cause of his/her condition remains irreversible, e.g. in cases of patients at the terminal stage of an irreversible disease which inevitably leads to death and the patient dies, despite application of modern ways of treatment. The restricted potential for cure places the staff in a situation, in which apart from challenges linked to therapeutic activities ethical dilemmas appear, stemming from the necessity to con-

sider stopping invasive methods of treatment and to diagnose terminal period of life. The situation in which such a diagnosis begins to be considered induces stress and anxiety both in the medical staff and in patient's family [7]. Due to his/her condition and due to applied invasive technologies, e.g. intubation or mechanical ventilation, the patient him/herself has no chances to undertake the decision self-standingly. In view of the specificity of the ICU one of the most challenging in diagnosing EOL in the uncertainty related to choice of most appropriate moment for informing patient's family on the unfavourable diagnosis [8–10]. Both nurses and physicians express apprehension of stripping off patient family's hopes by informing them on the diagnosis. Studies indicate that medical staff, including nurses, are no adequately trained in this respect [11, 12]. Moreover, the care provided in ICU in end-of -life frequently complicates the short time of passing away. Most of deaths in ICU occur within 4 h after diagnosis of EOL [13]. The risk of a sudden death does not allow „to prepare the patient for death", which is possible in cases of palliative patients. The nurses are confronted with an enormous challenge to provide an appropriate care to the dying patient and to his/her family [14]. With increasing frequency the need is stressed to assure the highest quality care to critically ill patients. In the care of patients quality of last moments in life is regarded more important than a heroic struggle for life prolongation [15].

Care of patient and of his/her family in the terminal period of life

Passing away represents a difficult period not only for a patient and his/her family but also for the staff taking care of the patient, it passes dynamically and the management should be modified, reflecting the changing and surprisingly variable condition of a patient and his/her close and dear. This requires that the care and symptomatic treatment are being appropriately adjusted and efforts are made to foresee apprehensions of patient's relatives [5].

In securing an effective continuity in care of patients in EOL, application of recommendations formulated in Liverpool Care Pathway (LCP) can be helpful. In their primary version, the recommendations were formulated at the end of 1990s, aimed to improve care of patients at EOL. They were worked out by employees of a hospice in Liverpool together with physicians of Royal Liverpool and Boradgreen University Hospitals, originally for needs of oncological patients. LPC assumes that a physician and nurses in common have to admit that there are no chances for improvement of health condition in a severely ill patient. Consequently, in such patients administration of drugs prescribed for a given disease and invasive treatment are discontinued. The situation should be discussed with family of the patient and, if possible, with the patient him/herself. Condition of the patient should be evaluated every four hours. If improvement is noted by the medical staff, the traditional treatment should be reinstalled.

The determination if the patient is just about to die is important not only due to the potential to introduce an appropriate treatment but also because it provides the potential to inform the patient (if he/she wishes so) on advancement of the disease, so that he/she, being aware of the approaching death, will be able to choose an appropriate treatment in the terminal stage of life. LCP in ICU aims at improving care of patients in their terminal hours/days of life, by definition of clears aims of care on the basis of patient's bio-psycho-social condition [16]. For an appropriate functioning of the programme implementation of of appropriate training is indispensable, as well as introduction of LCP pathway in individual wards [17]. Unfortunately in cases of patients in ICU the EOL takes a very short period of life and it is not always possible to introduce its appropriate management [18].

In 2003, Clark et al. presented the concept of seven key factors which affect quality of care over patients in EOL. They included communication, recognition of the patient and his/her family as most important in undertaking decisions, continuity of care, emotional support, provision of an appropriate care, an appropriate symptomatic treatment, spiritual support, emotional and organizational support [19].

In the care of patients in EOL an important ability is observation of the patient in the entirety of his/her biological-psychic-spiritual-social needs. Due to an inquisitive observation we can detect alterations and needs of the patient, with whom we frequently are unable to communicate verbally.

Communication with a dying patient represents an important art., in which a nurse plays a key role [20]. Laurette et al. suggest that an appropriate communication in ICU forms a groundwork of care in patients at EOL [4]. Most of the patients cannot properly communicate with either family or medical staff due to their health condition. We can hardly understand the language of dying persons and guessing the significance of the very restricted non-verbal transfer of informa-

tion (grimaces, groans, cry, repeated movements of an extremity, signs given by movements of the head, lips, eyeballs, blinking), even if difficult in interpretation, represent an important, undervalued system of communication [5]. The extraverbal communication provides base for nursing practice [20].

With approaching death of a patient much more attention should be devoted to support of his/her close and dear, who take care of the patient. In the care of the patient in ICU and of his/her family it should be kept in mind to appropriately transfer information and to be aware that the family may hear divergent data and communicates, both formal and informal ones related to health condition of the patient from several members of the therapeutic team. Due to extensive caretakers changes (work in relays) it may happen that the family receives erroneous communiques [21]. Studies demonstrated that family members of critically ill patients manifest high level of anxiety and fear, resulting from an insufficient communication with medical staff and lack of space (accommodation for the family) [22]. The level of fear is elevated also by the patient's surrounding itself (medical equipment, numerous leads, sound signals, alarms coupled to the medical equipment). Bach et al. point to a significant role of a nurse in providing emotional support to the family, resulting just from staying by the patent's bed or from conversation with family members [23].

It is common that patients in EOL suffer from pain [24]. It is frequently linked to execution of nursing procedures, such as change in position, aspirations or change of dressing. Pain alleviation in patients in EOL in ICU may be very difficult due to the applied sedation, which impedes evaluation of the pain. In cases of patients under sedation it is recommended to evaluate pain according to Behavioural Pain Scale and Critical-Care Pain Observation Tool [25, 26]. The alleviation of pain involves administration of opioids and anxiety-relieving drugs. In cases of using sedative drugs in patients in TPL frequently the principle of double effect in mentioned: administration of the drugs favourably alleviates suffering but unfavourably strips the patient of his/her awareness and the potential for abbreviation of life. Intervention which induces serious, anticipated unfavourable effects, including death remains morally admissible if undertaken with the intention to help the patient and unfavourable action represented a condition for obtaining a favourable effect. An unfavourable proved to be use of the „terminal sedation" term, which appeared in publications related to use of sedation in EOL. Application of sedation should not be mistaken with euthanasia. A deep, permanent sedation is used very seldom and it used to be indicated in irreversible and advanced disease with expected death within the approaching hours or days. Use of sedation in high doses frequently proves indispensable in last hours of patient's life, in order to prevent discomfort, to alleviate pain and asphyxia even in a situation when such actions are linked to an increased risk for abbreviation of patient's life. The use of sedation in EOL always should assure comfort to the patient [27]. In such situation no clinical need arises to increase body hydration; such activity might augment the risk of „premortal rattling", pulmonary oedema and peripheral oedema.

If there exist no doubts that the patient will die within days (e.g., in a neoplastic disease with metastases) an increased body hydration may harm the patient due to an increased amount of exudate in bronchial tree, providing no additional survival benefit and extra alleviation in suffering. In patients following a widespread stroke, who are unable to swallow but will survive perhaps longer than a few days, renal insufficiency develops and a pronounced thirst, if they are not extra-intestinally administered with fluids. They have to be rehydrated even of such procedure is thought to be pointless Studies indicate that therapy with fluids and alimentation not always make the patient comfortable [28].

In patients who die or begin to die frequently a troublesome anxiety or excitation develops. At this stage of a disease communication with the patient is reduced or impossible. The only practical manner of relieving the symptoms involves application of a certain stage of sedation but the decision on necessity of administration sedation-inducing drugs can be undertaken exclusively by the medical staff with an appropriate knowledge and experience [29].

Scientific data prove that an effective relief of symptoms in EOL frequently results in abbreviation of life [3]. Tokarz, in studies conducted in ICU in Poznań, found that in opinions of 98% studied medical staff the care for comfort in terminal moments of life is important and only 2% of the staff heroically fights for life prolongation by every minute [15].

Involvement of a nurse in undertaking decisions related to a patient in EOL

In the context of care over patients in EOL in ICU, discontinuation of intense treatment pertains to the till now conducted treatment and undertaking no subsequent therapeutic initiatives. In Poland this topic con-

tinues to represent a taboo, infrequently discussed but frequently erroneously associated with euthanasia.

However, it should be remembered that the treatment obligation does not extend to the phase of the dying patient while medical interventions which prolong agony represent an unethical activity, breaching patient's right to a dignified death. Artificial elongation of an agony needlessly increases patient's suffering, suffering of his/her close and dear and negatively affects the therapeutic team. In daily hospital discussions the lack of acceptance for resuscitation procedures, undertaken, e.g., in cases of agony in terminal neoplastic disease, is clearly articulated. In European recommendations of 2004 it was agreed that a superior aim of persons participating in decisions in TPL should involve patient's benefit. The recommendations indicate that nurses should play a significant role in activities of the therapeutic team [30]. In studies conducted by the European Federation of Critical Care Nurses (EfCCNa) in 2005, presented at the congress of anaesthesiology nurses it was demonstrated that 145/158 (91.85%) interviewed nurses were engaged in care of patients in EOL while 73.4% (n = 116) of them took share in undertaking decision on stopping persistent therapy [31]. 63.5% of the interviewed nurses initiated a discussion on diagnosing EOL in a patient. Most of the interviewed nurses (58.7%) expressed the opinion that involvement of nurses in diagnosing EOL is indispensable for an effective communication between a physician and patient's family [32]. The role of a nurse involves, first of all, provision of an emotional support for the family. Due to her/his frequent contact with close and dear of the patient, the nurse develops a confidence required in relations with families of dying patients. A professional, based on reciprocal respect cooperation between nurses and physicians promotes provision of support to the families. Studies conducted in USA indicated that an improper collaboration between nurses and physicians in care of a patient in EOL was linked to a more pronounced suffering of patient's family and lower professional satisfaction of nurses. Authors of the study stressed that accord between the two professional groups in diagnosing EOL is favourable for the dying patient and his family [33]. The final decision on discontinuation of persistent treatment remains the responsibility of physicians even if it is accepted that diagnosis of EOL should be based on condition of the patient, opinions of the family and medical staff and occasionally the consensus cannot be achieved [34–36]. It remains important that standards related to EOL are uniform [37–39].

In Poland undertaking the decision on implementation or abandoning resuscitation measures (DNAR – Do Not Attempt Resuscitation) represent still a taboo topic, reluctantly officially considered and, therefore, in awareness of several physicians erroneously associated with euthanasia. It occurs that saving measures are undertaken in situations forecasting no success and even when the patient provided no consent for starting such activities. In Polish hospitals decisions on abandoning resuscitation seldom are being documented [40]. The decision on not undertaking resuscitation in cases of circulatory or respiratory arrest is undertaken by physician who is in charge of the therapy. Before undertaking such a decision consultation with another physician is advisable, a physician who might help in an objective evaluation of the situation as well as an attempt to establish will of the patient related to undertaking resuscitation attempts. It would be advisable also to discuss the matter with the patient's relatives. In course of the conversation with the family it should be stressed that the final decision is undertaken by the physician and shifting the responsibility to patient's relatives makes no sense and is unjust. In Polish hospitals frequently patients are fully excluded in discussion related to his/her fate and role of his/her family becomes minimised. Such a practice breaches patient's right for autonomy and breaches the principle of expressing conscious consent for the manner and range of treatment, resulting from the paragraphs of the penal code, Act on Profession of a Physician and the Code of Medical Ethics [41, 42]. Role of the patient and his/her family in the process of establishing the range of therapy in critical conditions is radically different in individual countries. In USA autonomy of the patient represents a priority and, therefore, key decisions related to treatment, including management of critical conditions are undertaken with his/her involvement. In Europe, the range of medical management more frequently is discussed with the family (77%) than with the patent him/herself (26%) [34]. In Poland, the nurse taking care of the patient is not considered by interviewed physicians as a partner and a full standing member of the medical team. Therefore, nurses' opinion on selection of the manner of treatment in severely ill patient is not taken into account and, moreover, physicians see no reason to alter this type of practice [40]. Cause of the phenomenon seems to stem from work organization in hospitals. Staff deficiencies cause that every nurse cares for an excessive number of patients and, therefore, a nurse remains for every of the patient a foreign and anonymous person. Consequently, no premises exist to include the nurse to such a dif-

ficult discussion. On the other hand, in France nurse staff participates in 54% in decisions on reduction of therapy [44]. In Portugal, 35% physicians see the need for including nurses to the team deciding on resuscitation [44]. A similar opinion is expressed by 30% Australian physicians and 36% patients [45].

Support of medical staff in the course of its care over patients in EOL

Care of patients in EOL is associated with enormous stress to the nurse staff [46–48]. Its awareness of mastered capacities and satisfaction from professional work can become impaired, a sensation of a guilt, imperfectness and failure may appear. This becomes evident particularly in younger members of the team. Lack of support in such situations may induce restrain of sadness and anxiety, resulting in a mounting stress [49]. In questionnaire studies conducted by Sleziona and Krzyżanowski among 120 nurses a decisive majority of participants argued that they experience problems in certain aspect of care involving dying patients and require a support (e.g. by the close and dear) to cope with surviving such problems [50]. This points to the need for an appropriate education and self-improvement in the range of psychology in order to recognize reactions of a patient and his/her family. This is also significant for more accurate recognition of own personality traits and development of ways of how to neutralize effects of exposure to difficult situations [51].

Such an event should be discussed with the team using techniques of constructive and positive criticism and the persons in need should be suggested to take advantage of psychologist's support. The way of discussing the matter should be carefully adjusted to the needs: this may involve an informal talk or professionally conducted psychological consultation. The grief following experiencing death at work may represent normal reaction to the non-standard situation. This occurs particularly frequently in a situation when we deal with death of a young, till now healthy person, who within few hours or days entered EOL, e.g., due to a rupture of cerebral aneurysm. According to several authors, there exists a real need for mourning following death of a patient [49].

Summary

Care of patients in EOL is linked to enormous stress for the staff taking care of the patient and his/her family. The moment of undertaking a decision on discontinuation of an intense treatment or giving up resuscitation measures poses a problem and, therefore, it is important to unify standards related to EOL. Educational activities should be supported, which broaden knowledge on EOL since this will allow to assure the possibly highest quality care of patients in EOL and his/her family.

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Correspondence address:
Sylwia Miętkiewicz
Zakład Pielęgniarstwa Anestezjologicznego
i Intensywnej Opieki
Uniwersytet Medyczny im. Karola Marcinkowskiego
w Poznaniu
Wydział Nauk o Zdrowiu
11 Smoluchowskiego Str., 60-179 Poznań, Poland
phone: +48 503531664
e-mail: sylwiamietkiewicz@interia.pl

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1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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