**Introduction**

Wounds are unavoidable events of life, and their treatment is a most important medical problem. Surgical interference is the most effective method of wound treatment, and includes debridement and further renewal of skin cover by autodermoplasty (ADP).

One serious complication of ADP procedures is skin graft failure, leading to the uncovering of previously closed wounds, graft loss, and an increased wound surface owing to the donor zone. Numerous factors are involved that may affect the results of the ADP procedure. When planning the terms of surgical interference, specialists have to take into consideration criteria regarding the visual readiness of the wound and the patient’s objective state.

**Aim.**

We studied the possibility of using concentrations of trien conjugates, Schiff bases, and ceruloplasmin in the plasma of patients with deep local wounds as markers of the risk of autoskin graft failure. Methods. Ninety patients with local wounds of different origin and age were studied. Results and conclusions. It was found out that the amounts of trien conjugates, Schiff bases, and ceruloplasmin in plasma before the operation helped to predict the risk of autoskin graft failure with a high degree of probability (up to 92%). We established optimum cut-off points for trien conjugates, Schiff bases, and ceruloplasmin as indicators of the results of autodermoplasty operations, i.e. 350 mg/l, 0.35 units of oxidation indices (UOI), and 0.05 UOI, respectively.

**SUMMARY.** Background. Surgical interference which includes debridement and further renewal of skin cover by autodermoplasty (ADP) is the most effective method of burn wound treatment. Skin graft failure is a serious complication of ADP procedures leading to the opening of previously closed wounds, graft loss, and an increased wound surface owing to the donor zone. Numerous factors are involved that may affect the results of the ADP procedure. When planning the terms of surgical interference, specialists have to take into consideration criteria regarding the visual readiness of the wound and the patient’s objective state.

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of using the concentrations of trien conjugates, Schiff bases, and ceruloplasmin in the plasma of patients with deep local wounds as markers of the risk of autoskin graft failure.

Methods

Subjects

Ninety patients (male, 59 / female, 31; age, 20-58 yr) with local wounds of different origin and age (from 3 days to 12 yr) were enrolled in the study. All the patients had been hospitalized at the Gomel Regional Burn Center, Republic of Belarus. Of the 90 patients studied 55 had local burn injuries (II-III A-B, IV) in the body and the lower and upper extremities, with a total body surface area burned ranging from 0.4 to 7% (area of deep burn, 0.03 to 4%), 26 had post-traumatic or necrotic wounds and nine had trophic ulcers following chronic insufficiency of leg vein blood circulation or scarring. None of the patients had any serious associated pathology. After pre-operative treatment that included the use of bandages with antiseptic solutions and ointments on a polyethylene glycol base, all the patients were subjected to necrectomy and ADP procedures. As pre-medication, solutions of dimedrol and analgin were used. Surgery was carried out under general anaesthesia. From the surface of the femur or forearm, split-thickness (0.4 mm) free skin graft or grafts were taken with a dermatome and punctured. For complete closure the surface of dermal layer from the handle of the scalpel, we obtained the material, which we put on laboratory glass, fixed with 96% ethyl alcohol (for 2 min), and stained with haematoxylin and eosin (for 15 min). The smears obtained were analysed to determine the content of the following cell elements: bound nuclear and segmented neutrophils, phagocytes and degenerative forms of neutrophils, eosinophils, lymphocytes, monocytes, histiocytes, macrophages, fibroblasts, fibrocytes, and endothelium. The results were expressed in percentages per hundred of counted cells.

Biochemical assay

• LP processes

All the chemicals used in the present study were an analytical grade from standard Russian sources. The intensity of LP processes was measured using TC and SB levels (Volchegorsky and Lvovskaya method). Plasma (0.5 ml) plasma was added to 5 ml heptane-isopropanol (1:1 vol/vol). This mixture was vigorously shaken for 15 min and cleared by centrifugation (15 min, 2000 g). The lipid extracts were then mixed with 5 ml heptane-isopropanol (3:7 vol/vol) and 2 ml HCl (pH, 2.0) and allowed to stand for 30 min, after which the heptane layer was aspirated. In the isopropanol phase of lipid extracts we added 1 g NaCl for drying purposes and we left to stand for 30 min. Absorbance of the lower isopropanol layer was measured at wavelength 220, 278, and 400 nm against n-isopropanol on a spectrophotometer (SF-46 Lomo, Russia). Finally, the concentration of TC and SB in the isopropanol phase was calculated as an E278/220 ratio (amount of TC) and an E400/E220 ratio (content of SB) and expressed in units of oxidation indices (UOI).

• Ceruloplasmin

The ceruloplasmin (CP) concentration in the plasma was determined using the Ravin method. The CP level was measured spectrophotometrically at a wave length of 550 nm. The assay mixture contained 0.1 ml serum, 8 ml of 0.4 mol/l acetate buffer (pH, 5.5), and 1 ml of 0.5% p-phenylenediamine (Sigma). The mixture was heated in a hot water bath at 37 °C for 60 min. The control sample contained the same components but the first 2 ml of NaF were added before incubation. Prior to cooling at 4°C for 30 min, we added 2 ml of 3% NaF to the examined sample. The CP level in the blood was expressed in mg/l of blood. The calculations were made using the formula: absorbance of examined sample at wavelength 540 nm against control 875.

• Cytological evaluation

The wound cytograms of 57 patients were studied. In the course of the operation, after necrectomy, we carried out surface biopsies of the wound centre (Camaev technique). Having taken a light scrap of the wound surface layer from the handle of the scalpel, we obtained the material, which we put on laboratory glass, fixed with 96% ethyl alcohol (for 2 min), and stained with haematoxylin and eosin (for 15 min). The smears obtained were analysed to determine the content of the following cell elements: bound nuclear and segmented neutrophils, phagocytes and degenerative forms of neutrophils, eosinophils, lymphocytes, monocytes, histiocytes, macrophages, fibroblasts, fibrocytes, and endothelium. The results were expressed in percentages per hundred of counted cells.

• Statistical analysis

The statistical analysis was carried out using the statistical package SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). The results are presented as mean ± SE. The nonparametric Mann Whitney-U test was used.
to compare the two independent groups. The Wilcoxon test was used to compare dependent samples (variables). Correlations were analysed using the Spearman test. The results were considered statistically significant at $p < 0.05$.

We used an additional statistical method (logistic regression) as a way of describing the relationship between risk factors (predictors), such as concentrations of TC, SB, and CP in plasma, and an outcome of the ADP procedure (which has only two possible values: skin graft failure or complete graft take) to predict the result of the procedure. It is known that logistic regression allows one to predict an outcome from a set of variables. An explanation of the logistic model begins with an explanation of the logistic function:

$$P = \frac{1}{1 + e^{-\gamma}}$$

The variable $\gamma$ represents the exposure to some set of risk factors, while $P$ represents the probability of a particular outcome, given that set of risk factors. The variable $\gamma$ is usually defined as follows: $\gamma = a + b_1 \cdot x_1 + b_2 \cdot x_2 + \ldots + b_n \cdot x_n$, where $a$ is the “intercept” and $b_1, b_2, b_n$ and so on are the “regression coefficients” of $x_1, x_2,$ and $x_n$ respectively.\textsuperscript{16}

We used ROC (receiver operating characteristic) curve methods to determine cut-off points on the predictors (concentration of TC, SB, and CP in plasma) when the response variable had been analysed by logistic regression. It is known that the ROC curve is a graphic plot of sensitivity vs. (1 - specificity) for a binary classifier system as its discrimination threshold is varied.\textsuperscript{17,18} To estimate the quality of the models obtained we valued the area under the ROC curve (AUC).\textsuperscript{19}

**Results**

Table I contains data regarding the distribution of patients with various post-operative courses in relation to their age, size of wound, and aetiology of wound injury.

As is clear from Table I, the group of patients with a favourable post-operative course (Group 1) and the group with subsequent rejection of skin grafts (Group 2) were comparable by age, size, and origin of wound injury. The difference in parameters between the groups was statistically negligible and the groups can therefore be considered to be comparable.

**Cytological results**

None of the patients’ wounds presented any symptoms of inflammation (secretion, blushing), but showed elevated adhesiveness and marginal epithelialization. Our analysis of the patients’ cytograms took into consideration wound duration (acute and chronic) and the main features of the post-operative periods (Group 1 - complete graft take, n = 25; Group 2 - skin graft rejection, n = 21). The cytological composition varied, depending on wound duration, but none of the patients showed any sign of festering (which means there were no necrotic or degenerative types of cytogram).
Table II presents the results of the cytological study and comparative data regarding wound duration and the post-operative period.

Thirty-two patients with acute wounds (up to 2 months after wounding) demonstrated regenerative types of cytograms. As Table II clearly shows, the number of neutrophils of different types decreased (less than 30%), Mononuclear cells forming granulative tissue - fibroblasts and fibrocytes (15-47%) - prevailed. Endothelium and plain epithelium cells occurred. Fourteen patients with chronic wounds, treated surgically more than two months post-injury, presented more neutrophils of different types in wound bioplates (40%) than patients with acute wounds, but vivid signs of regeneration manifested themselves (macrophage, fibroblast, and fibrocyte content was the same as in cases with acute wounds). The data show that in general there were no significant statistical differences between the cytograms of patient wounds in Group 1 and those of Group 2 except in one regard; patients in Group 1 presented eosinophils (average, 2.5 ± 0.2) in their wound cytograms but not in Group 2.

Thus, on the basis of the clinical and cytological criteria, all the patients were completely ready for autograft closure.

Levels of the TC, SB, and CP in the plasma of patients with local wounds

Table III presents the mean values of TC, SB, and CP in the plasma of healthy adult individuals and of the wounded patients prior to surgery.

As is clear from Table III, there were significant essential increases in the plasma levels of TC, SB, and CP in patients with local wounds before surgery in comparison with healthy individuals ($p < 0.001$, $p < 0.01$). This indicates that the local injury activate the general process of accumulation of LP products and antioxidants in the blood. Prior to ADP procedures, patients in Group 1 and Group 2 demonstrated increased TC, SB, and CP concentrations in plasma in comparison with healthy individuals ($p < 0.01$, $p < 0.001$) (Table III). The extent of the increase depended on the course of the post-operative period. Group 2 patients (skin graft lysis in post-operative period) presented much lower TC and SB concentrations in the plasma ($p < 0.01$) than Group 1 patients (favourable surgical outcome). The CP concentration values varied analogically. Patients with a favourable outcome of the ADP procedure (Group 1) presented a CP concentration that was almost twice as high as in Group 2 patients and 64% higher than healthy individuals (Table III). Group 2 patients had only a 13% increase in CP concentration.

Analyses of data in the different groups of patients demonstrated a positive and significant correlation between increased TC, SB, and CP levels in plasma before surgery and a favourable outcome of the ADP operation (respectively, $r = 0.51$, $p = 0.028$, $r = 0.55$, $p < 0.0001$, $r = 0.52$, $p = 0.002$).

The diagnostic value of TC, SB, and CP plasma levels as markers of the risk of ADP complications

Logistic regression enabled us to establish that the concentrations of TC, SB, and CP in plasma before ADP procedures make it possible to predict the risk of skin transplant failure in the course of wound treatment with a high degree of accuracy (percentage of correct predictions, approximately 92%).

Using ROC curves, a cut-off point was determined in the continuous variables, such as TC, SB, and CP concentrations in plasma. The value for CP was 350 mg/l (sensitivity = 97%, specificity = 83%), for SB 0.05 UIO (sensitivity = 91%, specificity = 81%), and for TC 0.35 UIO (70% and 84% respectively). For every parameter we obtained maximum proximity in the upper left corner of the ROC space, while remote curves gave a point along a diagonal line from bottom left to top right. AUC for CP was 0.911 (outstanding quality), for SB 0.845 (very good quality), and for TC 0.745 (good quality).

Thus, if prior to an ADP procedure the concentration values of TC, SB, and CP in plasma are lower than the determined cut-off point, skin graft failure can be predicted. But if the values are higher than the determined cut-off point complete graft take can be expected (the probability is respectively about 94 and 91%, respectively).

As we had some other correlations between the determined cut-off point and the predictors (for example, one

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration</th>
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<tbody>
<tr>
<td></td>
<td>Healthy adult individuals (n = 40)</td>
</tr>
<tr>
<td>CP (mg/l)</td>
<td>245 ± 12</td>
</tr>
<tr>
<td>TC (UIO)</td>
<td>0.214 ± 0.007</td>
</tr>
<tr>
<td>SB (UIO)</td>
<td>0.022 ± 0.008</td>
</tr>
</tbody>
</table>

Values are mean ± SE. * all patients with local wounds compared with the healthy controls ($p < 0.001$, $p < 0.01$). 1 = patients in Group 1 and patients in Group 2 compared with healthy adults ($p < 0.05$, $p < 0.001$). 2 = patients in Group 1 compared with patients in Group 2 ($p > 0.05$, $p < 0.001$).

UOI = units of oxidation indices
of the predictors was lower than the cut-off point, while others were higher than the cut-off point or other variants), we used a logit model.

This is the model we set up: \( a = 64.239 \) (the intercept), \( b_1 = -3.481, b_2 = -19.949, b_3 = -50.127 \); \( \chi_1 = \text{CP concentration}, \chi_2 = \text{TC concentration}, \chi_3 = \text{SB concentration} \).

For convenience sake we used codes instead of concentration values of TC, SB, and CP in plasma: 1. when the value was lower than the cut-off point; 2. when the value was higher than the cut-off point.

Thus: \( \gamma = 64.239 - 3.481 \times \text{code of CP} - 19.949 \times \text{code of TC} - 20.127 \times \text{code of SB} \).

We used the value of \( \gamma \) in a logistic function formula (see above).

A \( p \) value above 0.5 predicts skin graft failure, while \( p \) below 0.5 predicts successful grafting.

**Practical application of the results for the prediction of the outcome of ADP procedures**

The fifty-eight patients examined had higher concentration values of the plasma TC, SB, and CP than the cut-off point, and we therefore expected successful grafting. In fact, the outcome of the ADP operation was favourable in 100% of cases. The skin grafts took on day 2 or 3 and grafting was complete by day 7-9 post-ADP. The concentration of plasma TC, SB, and CP in the other 32 patients was lower than the cut-off point, and skin graft failure could be expected. These patients were however subjected to the ADP procedure, although in all cases the post-operative period was complicated. They received conservative treatment and regrafting, which prolonged their hospital stay.

**Discussion**

The majority opinion is that the surgical repair of skin defects by ADP using split-thickness autoskin graft is one of the most economical and effective method of wound treatment. Skin graft failure is a serious complication of ADP. The reasons for autoskin graft failure are overwhelmingly thought to be of a subjective and technical nature, and for this reason they should be eliminated by all available means. However, in spite of significant progress in surgical technique, cases of autoskin graft failure are still fairly frequent (up to 10-30%). This demonstrates the need to take into consideration the organism’s general capacity of reaction and the levels of the metabolic processes.

To determine the wound’s readiness for surgery, one should use not only visual clinical criteria but also some objective methods. One of the most popular methods is the cytological rating of the wound’s condition. In cases of acute or chronic wounds, regenerative cytogram types with insignificant signs of inflammation are considered to be optimal for surgery. In cytograms of this kind the number of lymphocytes and histiocytes increases with the background of a relative decrease of the neutrophil level, the active reaction of macrophages and fibroblasts, and the appearance of endothelium cells. Cytogram control in the course of acute and chronic wound operative treatment allows one to improve the outcome of treatment. According to our data, all patients with early ADP had successful post-operative results, although 35% of the patients (21 out of 57) with acute and chronic wounds subjected to operation against the background of minimal signs of inflammation (according to the cytogram data) were affected by skin graft failure. We found no difference between the pre-operative cytograms of patients with different post-operative courses. It is interesting to note that only patients with a favourable operation outcome presented eosinophils in their cytograms - patients with a complicated post-operative course had none. It is known that eosinophil enzymes (lysophospholipase, phospholipase, etc.) promote the elimination of cell remains by the mechanism of extra-cell cytolysis. However, the role of eosinophils in the repair processes continues to be uncertain.

The results of our research therefore did not provide any opportunity for predicting the outcome of an operation on the basis of cytograms in patients with acute and chronic wounds.

The development of adaptive processes was shown to be accompanied by an increase in reactive oxygen species, which play the role of signal molecules. In the meantime, the process of LP activation started - this altered membrane permeability and functions of ion channels and receptors.

We were able to show that the presence of a local wound defect was accompanied by lipoperoxide (TC, SB) accumulation, with a simultaneous increase of CP antioxidant in blood plasma. This correlated with the results of other researchers. We also found that pre-operative values of TC, SB, and CP concentrations in plasma differed from those of patients with different operation outcomes.

This allows us to suppose that these values may serve as predictors of autoskin graft failure. We estimated the probability of autoskin graft failure on the basis of supposed predictor values (concentrations of TC, SB, and CP) in plasma by the logistic regression method. It was found out that by determining the pre-operative values of TC, SB, and CP simultaneously it was possible to predict the probability of skin graft failure (in 94% of cases) and successful graft take (in 91% of cases), depending on the values obtained. In general, the percentage of correct predictions was not less than 92%.

For the purpose of practical application of the results obtained, we employed ROC analyses to establish the threshold values of the TC, SB, and CP concentration when the balance between test specificity and sensibility was maximum. Using the threshold values, the regression formula, and the logistic function, we applied our results to the prediction of the outcome of ADP procedures in 90 patients with local wounds. On this basis we predicted a success-
ful operation outcome in 58 patients. Our predictions proved accurate: all these patients achieved complete grafting. In the remaining 32 patients, using the criteria that we had worked out, we predicted complications after the ADP procedure, and in all 32 in whom the surgical outcome was predicted to be unfavourable, the predictions again proved to be accurate: these patients suffered skin graft failure.

RÉSUMÉ. Données générales. L’intervention chirurgicale qui comprend le débridement et le renouvellement ultérieur de la couverture cutanée moyennant autodermoplastie (ADP) constitue la méthode la plus efficace pour le traitement des lésions causées par les brûlures. L’insuccès d’une greffe cutanée est une grave complication des procédures de l’ADP qui porte à l’ouverture des lésions déjà fermées, à la perte du greffon et à l’augmentation de la surface éventée en raison de la zone donneuse. De nombreux facteurs contribuent aux résultats de la procédure de l’ADP. Au moment de la planification des modalités de l’intervention chirurgicale, il faut que les spécialistes prennent en considération des critères qui concernent l’aspect visuel de la lésion et la condition objective du patient pour décider s’il est prêt pour l’opération. Objectif. Nous avons étudié la possibilité d’utiliser les concentrations de conjugués de trien, des bases de Schiff et de la céruloplasmine dans le plasma des patients atteints de lésions locales profondes comme marqueurs de l’insuccès de l’autogreffe cutanée. Méthodes. Quatre-vingt-dix patients atteints de lésions locales d’origine et d’âges différents ont été étudiés. Résultats et conclusions. Il a été constaté que les quantités de conjugués de trien, des bases de Schiff et de la céruloplasmine dans le plasma des patients atteints de lésions locales profondes locales comme marqueurs de l’insuccès de l’autogreffe cutanée peuvent se démontrer utiles pour prédire le risque d’insuccès de l’autogreffe cutanée, avec un haut degré de probabilité (jusqu’à 92%). Nous avons établi des niveaux limites optimaux pour les conjugués de trien, des bases de Schiff et de la céruloplasmine comme indicateurs des résultats des procédures d’autodermoplastie, c’est-à-dire 350 mg/l, 0,35 unités des indices de l’oxydation (UIO), et 0,05 UOI, respectivement.

BIBLIOGRAPHY


Conclusion

The present study shows that trien conjugates, Schiff bases, and ceruloplasmin values in the plasma of patients with local wounds can serve as markers for the estimation of the risk of skin graft failure after autodermoplasty surgery.