Correlation of the Main Parametres of Immun Systeminchildren with Localized Scleroderma by the Stages of the Disease

Andrey Mun[1], Abdushukur Mannanov[2]

[1]Scientific Researcher, MD, Teaching Assistant, Department of Dermatological and Venereal Diseases, Pediatric Dermatological and Venereal Diseases and AIDS, Tashkent Pediatric Medical Institute, 223 Bogishamol Str., Yunusobod district, Tashkent, Uzbekistan, [2]Doctor of Medical Sciences, Professor, MD, Department of Dermatological and Venereal Diseases, Pediatric Dermatological and Venereal Diseases and AIDS, Tashkent Pediatric Medical Institute, 223 Bogishamol Str., Yunusobod District, Tashkent, Uzbekistan

Abstract

Introduction: In spite of significant development in modern medicine, the tendency of increase in the number of patients with localized scleroderma (LS) remaining actual with more severe clinical courses. Therefore, LS remains as one of the urgent problems of healthcare system. The etiology and pathogenesis of the disease is still unclear. Yet, LS frequently associates with deep and wide immune disorders and subsequent complications.

Materials and Method: We studied 45 children with LS, of whom 25 patients had a linear form, 16 had plaque form, Pazini-Pieriniatrophodermia – in 2 participants, scleroatrophic lichen in 2. The control group included 30 conditionally healthy children. We conducted monitoring of the cellular immunity system with immunophenotyping using specific monoclonal antibodies to surface molecules CD3, CD4, CD8, CD16, CD20, CD25, CD54, CD95. Determination of the main classes of immunoglobulins in serum - IgG, IgA, IgM was also carried out.

Results: There was a significant decrease in the number of T-helpers - CD4: at the stage of erythema and edema by 1.3 times, with sclerotic and atrophic - by 1.2 times. The content of cytotoxic CD8-lymphocytes significantly decreased at the stages of erythema, edema and sclerotic on average by 19.4%. As a result of more significant inhibition of CD8, the CD4/CD8 ratio became larger (by 24%, 25%, and 11%, respectively, at the stage of erythema, sclerosis, and atrophy) than in the group of conditionally healthy patients. Patients with LS, a statistically significant decrease in the serum percentage of total T-lymphocytes - CD3, and at the stage of erythema and atrophic edema it was 11.4%, and at the stage compaction - 6.3% compared with the parameters of control group.

Conclusion: According to the level of inflammatory process with LS, immune dysfunctions are associated with a decline in the total amount of blood lymphocytes, namely CD4 T-helper cells and CD8 T-suppressors, CD54 endotheliocyte adhesion molecule, especially in the stages of erythema and edema or sclerosis. Learning these parameters may help to assess the immunological changes, and can be informative for the determination of the course, prognosis and further treatment.

Keywords: *Morphea, localized scleroderma, children, cellular immunity, humoral immunity, interleukin.*

Introduction

Localized scleroderma (LS) or “morphea” is a progressive lesion of the connective tissue of the dermis and hypodermis, without involvement of internal organs in the process with a predominance of fibro-sclerotic and vascular disorders, such as obliterating endarteriolitis with widespread vasospastic changes. The disease develops on the background of genetically determined

imperfection of immunoregulatory processes, resulting in the formation of antibodies to own cells and their components, the occurrence of immunocomplex inflammation, leading to tissue damage (Mannanov & Moon, 2018; and Moon, 2019)[1].

Despite intensive studies during the last few decades, there is a tendency to increase the number of patients with LS with a more severe clinical course, thus, LS remains as one of the urgent problems of modern medicine[2]. The etiology has not yet been identified well, and the pathogenesis of the disease is also unclear[3]. The issues of clarifying the links in the pathogenesis of LS, determining diagnostic markers of the severity of the process, and substantiating new treatment method based on certain disorders remain as actual problem today (Moon, 2019; and Shostak et al, 2015)[4]. However, the main role in the development of scleroderma is played by 3 theories of pathogenesis, immune disorders, metabolic pathology and vascular disorders in the connective tissue. One of the main links in the pathogenesis of scleroderma is immune disorder (O'Brien et al, 2017; and Torok et al, 2015)[5]. However, the data on this issue are still disputable and require some improvements. It is important to evaluate the immune disorders in the blood, leading to morphological changes in the lesions by considering the course of dermatosis.

With localized scleroderma, pathological autoimmune processes develop with signs of activation of the T-cell link of the immune system, associated with ineffective suppressor mechanisms in the immune system, which are represented by cytotoxic T-lymphocytes and some other subpopulations of T- and B-lymphocytes.An increase in IL-2, IL-4, IL-6, and soluble IL-2 receptors in patients with limited scleroderma indicates activation of cellular immunity (Suzanne, 2018)[6].

Dysregulatory disorders are accompanied by increased production of pro-inflammatory cytokines IL-2, TNFα and INF-γ, contributing to the development of early autoimmune inflammation[7].There is no doubt that the pathogenesis of scleroderma is associated with a dysfunction of human immunity[8]. Evaluation of the immune status with diagnostic, therapeutic, prognostic and preventive purposes in patients with limited scleroderma remains an extremely urgent issue[9].

The aim of our work was to study the characteristics of the immune status of children with LS at different stages of the disease[10].

Materials and Method

Study Population: We studied 45 children with LS from January 2013 to December 2019, of whom 25 patients (55.5%) had a linear form, 16 (35.5%) had plaque form, Pazini-Pieriniatrophodermia–in 2 participants (4.4%), scleroatrophic lichen in 2 (4.4%). The degree of severity, characterized by a mild course, was observed in 12 children, II degree, moderate in severity - 22, III degree, which was characterized by a severe course - in 11.Of the total number of patients with LS in the abdominal and linear form, 16 (35.6%) patients were diagnosed with the stage of erythema and edema.In 18 patients (40%), the stage of sclerosis was diagnosed (I degree of severity - 4, II degree - 9, III degree - 5), 11 - stage of atrophy (I degree of severity - 4 people, II degree - 5, III degree - 2).

All patients with Pasini-Pieriniatrophodermia were in the stage of sclerosis (I severity). Patients with scleroatrophic lichen were in the acute stage of II severity and II severity. Thus, we studied children with LS at the stage of erythema and edema - 16 (35.6%), at the stage of sclerosis -18 (40%), atrophy - 11 (24.4%). The number of boys was 12 (26.6%), and girls - 33 (73.3%). The mean age of the examined was 6.8 ± 0.8 years.

Clinical and laboratory tools: The diagnosis of LS was verified and established according to clinical and laboratory data, and the severity according to the dermatological indices of the LoSCAT (Localized Scleroderma Assessment Tool) including mLoSSI (Modified Localized Skin Severity Index), LoSDI (Localized Scleroderma Damaged Index), based on an assessment of 3 separate processes - erythema, skin tightening, the appearance of new elements or an increase in existing ones during the previous month, the degree of atrophy of the skin and subcutaneous fat, as well as the severity of pigmentation (Zulian, 2017; and Teske & Jacobe, 2018)[11].

The control group included 30 conditionally healthy children who permanently reside in Tashkent city and the Tashkent region (18 girls and 12 boys, average age 7.4 ± 0.9 years).

Monitoring the state of the cellular immunity system was carried out by immunophenotyping using specific monoclonal antibodies to surface molecules CD3, CD4, CD8, CD16, CD20, CD25, CD54, CD95 of the "Scientific-Production Center" LLC (Moscow). The determination of the main classes of immunoglobulins in

serum - IgG, IgA, IgM, is the main method for assessing humoral immunity and their determination is of great diagnostic value in the diagnosis and monitoring of primary immunodeficiencies and autoimmune diseases. The content of immunoglobulins A, M, G in serum was determined by the enzyme-linked immunosorbent method "Immunoglobulins A, M, G - IFA". The concentration of circulating immune complexes was determined according to the method of Grinevich and Alferov (Mannanov & Moon, 2018; and Shostak et al, 2015)[12].

Immunocompetent cells were verified using "Chemicon" monoclonal antibodies (MCAs) (product of USA) in mature T-lymphocytes (CD3), T-helpers (CD4), T-suppressors (CD8), B-lymphocytes (CD22), macrophages (CD16), IL-1 and IL-2. Cells expressing receptors for HLA-Drantigen were determined using the MCA HLA-Dr antigen of the same company. The relative volumes of immunocompetent cells were determined by the aqueous field of view of the microscope in terms of 100 cells.

Results

Immunomorphological studies were carried out in 45 children with LS, of which 16 patients (35.6%) of the abdominal and linear forms were diagnosed with erythema and edema (I severity -5 people, II degree - 8, III degree - 3), in 18 patients (40%) - the stage of sclerosis (I degree of severity -4 people, II degree - 9, III degree - 5), in 11 patients (24.4%) - stage of atrophy (I degree of severity -4 people, II degree - 5, III degree - 2). The age of patients ranged from 2.5 to 17 years (average age was 6.8 ± 0.8 years), among whom there were 12 boys (26.6%), and girls - 33 (73.3%), the duration of the disease from 1 month up to 6 years.

When analyzing the obtained data of the immune parameters of the blood of children with LS, inhibition of the T-system of immunity was revealed - a statistically significant decrease in the serum percentage of total T-lymphocytes - CD3, and at the stage of erythema and atrophic edema it was 11.4%, and at the stage compaction - 6.3% compared with the parameters of control group.

There was also a significant decrease in the number of T-helpers - CD4: at the stage of erythema and edema by 1.3 times, with sclerotic and atrophic - by 1.2 times. The content of cytotoxic CD8-lymphocytes significantly decreased at the stages of erythema, edema and sclerotic on average by 19.4%. As a result of more significant inhibition of CD8, the CD4/CD8 ratio became larger (by 24%, 25%, and 11%, respectively, at the stage of erythema, sclerosis, and atrophy) than in the group of conditionally healthy patients.

The content of CD8-lymphocytes at the atrophic stage increased up to the level of control group. Despite the revealed disorders of the immune system, no statistically significant differences were found in patients with different numbers of lesions.

An increase in the immunoregulatory index indicated an activation of the immune system in the stage of edema and erythema, as well as in the stage of early sclerosis, however, a decrease in this indicator in the stage of atrophy and sclerotherapy in the later stages showed inhibition of immunity (Table 1).

Table 1: The content of lymphocyte subpopulations in the blood of patients with LS depending on the stage of the disease in children

Indexes	Control n = 30	Stage of the disease		
		Edema n = 16	Sclerosis n = 18	Atrophy, n = 11
CD3+,% (Overall pool of T-lymphocytes)	56,5 ± 1,3	42,0 ± 1,3	47,8 ± 2,2	41,4 ± 2,9
CD4+,% (T-helpers)	33,8 ± 1,4	22,3 ± 1,2	26,2± 1,1	27,1 ± 1,8
CD8+,% (T-citotoxic lymphocytes)	22,6 ± 0,6	15,2 ± 1,8	16,3 ± 1,4	25,0 ± 1,7
CD4/CD8 (immunoregulation index)	1,8 ± 0,04	2,47 ± 0,22	2,27 ± 0,27	1,37 ± 0,18
CD16+,% (natural killers)	14,5 ± 0,8	18,5 ± 1,7	19,3 ± 2,6	19,9 ± 1,5
CD20+,% (B-lymphocytes)	20 ± 0,7	24,5 ± 0,5	25,3 ± 2,5	26,0 ± 1,0
CD25+,%	7,4 ± 0,5	15,4 ± 3,0	18,1 ± 0,5	16,5 ± 1,0
CD95+,% (factor of apoptosis)	17,2 ± 0,5	22,3 ± 1,3	26,8 ± 1,0	24,1 ± 1,5

Indexes	Control n = 30	Stage of the disease		
		Edema n = 16	Sclerosis n = 18	Atrophy, n = 11
CD54+,% (factor of adhesion)	23,4 ± 1,1	14,2 ± 1,2	11,8 ± 1,0	17,1 ± 1,8
Leucocytes, $x10^9$/l	5,98 ± 0,27	6,45 ± 0,42	6,71 ± 0,38	6,64 ± 0,33
Lymphocytes, $x10^9$/l	2,26 ± 0,19	2,05 ± 0,16	2,16 ± 0,15	2,63 ± 0,11

It was important that significant decrease in the blood serum of patients with respect to the content of intercellular adhesion molecules, which are surface antigens of activated B-lymphocytes-CD54, namely, 36% at the stage of erythema and edema, 38% at sclerotic, 28% at atrophic stages of the disease compared with the control group. The content of adhesion molecules of endothelial cells CD54 at the atrophic stage of the disease remained significantly increased compared with the sclerotic stage by 41.7%.

It should be noted that the number of CD95 + cells responsible for apoptosis in sick children at the stage of atrophy increased by 27.2%.Especially statistically significant differences in the number of lymphocytes of different phenotypes were found for CD16, CD25 and CD95, the content of which was increased compared with conditionally healthy patients.

The stages and nature of the immunological process reflect the expression data of activation markers that depend on the functional activity of immunocompetent cells. Interpretation of the expression of active markers on the surface of lymphocytes showed a significant increase in apoptosis processes, in patients with LS as evidenced by an increase in cells carrying apoptosis receptors - CD95, the most pronounced increase was at the sclerotic stage - by 57.6% compared with the control group.

The relative content of CD95 in the stages of edema and atrophy was 31.2% and 41.7%, respectively. At the same time, an increase in the expression of receptors for CD95 testified to a significant destruction of lymphocytes, which led to immunodeficiency, a decrease in the number of lymphocytes, inhibition of T-suppressors and an increase in circulating immune components (CIC)in the blood.

In the examined patients, the content of markers of the early stage of cell activation, IL-2 CD25 activators increased by 2.1 and 2.4 times in the cases of erythema and edema and atrophic, respectively, and 2.2 times in the sclerotic stage compared with the control. As you know, IL-2 is a pro-inflammatory cytokine, which leads to inflammation, the appearance of severe vasculitis. Its level remained high throughout the entire disease, which led to a chronic process, activation, and the appearance of a significant pool of proliferating lymphocytes in LS. The amount of natural killers - CD16 increased on average by 35.2%, a change in the content was especially pronounced during the sclerotic and atrophic stages. A significant increase in the percentage of activation markers of the B-cell chain of the CD20 immune system was 22.5%, 26.5%, 30%, respectively, at the stages of erythema and edema, sclerotic and atrophic, compared with the control group.

The dynamics of changes in the lymphocyte phenotype was observed in the sclerotic and atrophic stages of the OS against the background of an increase in the total number of leukocytes and a relative decrease in lymphocytes. A decrease in the number of T-lymphocytes with an imbalance of subpopulations against the background of the development of activation processes and their increased tendency to apoptosis probably leads to a loss of control of T-lymphocytes in relation to B-cells, with their subsequent activation.

Our results indicate a significant increase in blood levels of IgM and IgG in the examined LS patients compared with the control against the background of insignificant changes in the content of IgA. The most pronounced was an increase in IgG, the content of which increased by 24.3%, 15.1% and 39.8%, respectively, at the stages of edema, compaction and atrophy.

In children with LS in the blood serum, an increase in the level of large and small circulating immune complexes of the CIC by 47.4% and 26.9% in the sclerotic and atrophic stages of the disease, by 63.9% in the stage of erythema and edema compared with the control (table 2). The production of antibodies, including autoantibodies, is controlled by the corresponding cytokines.

Table 2: The main indicators of the humoral immunity in the blood serum of children with LS

Indexes	Control, n = 30	Stage of the disease		
		Edema n = 16	Sclerosis, n = 18	Atrophy, n = 11
Ig A, g/l	1,82 ± 0,11	1,93 ± 0,14	1,71 ± 0,09	1,74 ± 0,11
Ig M, g/l	1,4 ± 0,07	2,01 ± 0,05	2,1 ± 0,04	1,93 ± 0,07
Ig G, g/l	9,73 ± 0,32	12,1 ± 1,77	11,2 ± 1,48	13,6 ± 1,29
CIC, y.e.	38,2 ± 3,3	62,6 ± 8,4	56,3 ± 9,2	48,5 ± 4,6

In the blood serum of patients with LS in the stage of edema, a significant increase in the content of IL-1β was detected on average 4.9 times, IL-4 2.05 times and IL-6 8 times compared with the control. In the sclerotic stage, these indicators increased by 3.7 respectively; 1.93 and 5.7 times; at the atrophic stage - 5.7; 1.7 and 3 times (table 3).

Table 3: The main indicators of the content of cytokines in the blood serum of children with OS, depending on the stage of the disease

Indexes	Control, n = 30	Stage of the disease		
		Erythema and edema, n = 71	Sclerotic n = 47	Atrophic, n = 37
IL-1β	5,68±2,12	27,92±3,12	21,1±2,38	32,41±2,83
IL-4	5,75±2,05	11,81±1,23	11,07±1,24	9,93±0,9
IL-6	5,71±1,7	45,53±6,09	32,32±5,23	17,42±1,93
TNFα	2,85±0,63	11,36±1,2	9,74±1,61	8,18±1,52
IFN-γ	273,3±28,9	95,8±9,1	78,5±8,2	168,3±12,1

Discussion

IL-1β, which is a pro-inflammatory cytokine, has numerous common effects and can contribute to the development of the systemic nature of the pathological process by forming autoantibodies and increasing the concentration of CRP in the blood. It should be noted that IL-4 and IL-6 are one of the frequently determined cytokines in the blood serum of patients with scleroderma. (O'Brien et al, 2016; and O'Brien et al, 2017).

In addition to B-lymphocytes, monocytes, macrophages and fibroblasts are also sources of IL-6 in scleroderma (Toroket al, 2015; and Suzanne, 2018). IL-6 is involved in the induction of almost the entire complex of local manifestations of inflammation. It affects the migration of phagocytes, enhancing the production of chemokines that attract monocytes and lymphocytes, and weakening the production of chemokines that attract neutrophils. The pro-inflammatory effects of IL-6 are less pronounced than that of IL-1 and TNFα, in contrast to which it does not enhance, but inhibits the production of pro-inflammatory cytokines (IL-1, TNFα and IL-6) and chemokines by cells involved in the inflammatory process (Toroket al, 2015). Thus, IL-6 combines the properties of pro- and anti-inflammatory cytokines and is involved not only in the development, but also in limiting the inflammatory response (Speeckaert et al, 2016).

Cytokines play an important role in the regulation of all aspects of the functioning of the immune system, including the differentiation of lymphoid cells, inflammation, the development of the adaptive function of the immune response, and others. One of the most important regulators of reactions of both non-specific and adaptive immune responses is TNFα and IFN-γ. TNF is produced both by cells of the immune system (B and T lymphocytes, basophils, eosinophils, dendritic cells, nK-cells, neutrophils) and other types of cells (astrocytes, fibroblasts, glial cells, keratinocytes), however, the main producers are monocytes and tissue macrophages (Toroket al, 2015; and Florez-Pollack et al, 2018).

IFN-γ is synthesized by activated antigen-specific T-lymphocytes (CD4 + th1 type). Interferon-γ has a strong immunoregulatory effect and occupies one of the central places in the regulation of adaptive immune response (Florez-Pollack et al, 2018; and Suzanne, 2018). In the blood serum of patients with LS, an increase in TNFα content was observed compared with the norm, and it was most pronounced at the stage of erythema and edema of the disease by 3.9 times, its content was slightly lower at the sclerotic and atrophic stages - at 3.4 and 2.9 times, respectively, compared with the control.

Another dynamics was observed for the content of IFN-γ, in the form of a significant decrease of 2.9 times at the stage of erythema and edema, 3.5 times at the sclerotic stage and 1.6 times atrophic compared with the parameters of the group of conditionally healthy patients. Perhaps a decrease in the synthesis of IFN-γ is associated with a low content in patients with LS of their main producers of CD4 lymphocytes.

Thus, our results indicate that the leading link in the pathogenesis of LS is a significant change in the indicators of humoral and cellular immunity. In the studied stages of LS, against the background of an increased tendency of blood lymphocytes to apoptosis, there is a loss of control of T-lymphocytes in relation to B-cells, with their subsequent activation and a significant change in the quantitative and qualitative composition of the lymphocyte subpopulation. These changes are accompanied by immune disorders in the skin.Histological examination of the skin in the erythematous stage as a manifestation of acute vasculitis revealed volumetric intradermal lymphocytic infiltrates.A significant increase in the number of lymphocytes indicates the activation of the function of the mononuclear phagocyte system in response to the destruction of all elements of the dermis (Walker et al, 2017).

In the sclerotic stage of the disease, the total number of mature lymphocytes remained at the levels detected in the erythematous stage, while the number of macrophages tended to decrease.Such dynamics testified to a decrease in antigenic stimulation in the body, which was combined with the attenuation of alternative exudative processes, the beginning of the development of sclerotic processes in the papillary and reticular layers of the dermis, and partial atrophy of the skin appendages, especially hair follicles and sebaceous glands (Walker et al, 2017; and Florez-Pollack et al, 2018).A decrease in the number of IL-1 producing cells with a decrease in the number of lymphocytes indicates a subsidence of the inflammatory process in the skin and a decrease in the secretion of neutral proteases - collagenase and elastase, which stabilizes the structure of connective tissue and inhibits further collagen destruction.

Together with a decrease in the total number of T-lymphocytes and their varieties in the sclerotic stage, a significant change in the number of macrophages was detected, which was associated with the disposal and elimination of skin tissue destruction products. The decrease in the number of cells of the T-system of immunity compared with their content in the previous stage was consistent with the data of a histological study of biopsy samples of the skin of patients with LS in the sclerotic stage, when, against the background of pronounced sclerosis of the dermis, hypodermis and atrophy of the epidermis, signs of immune inflammation are either absent or weak with the exception of individual cases requiring a correct assessment of the nature of the process and the correction of conventional therapy.

In a histological examination of the atrophic stage of LS against severe sclerosis, developing hyalinosis, reduction of blood vessels and appendages of the skin, the extinction of alternative-destructive and inflammatory processes, the number of total T-lymphocytes reached low values, but did not decrease to normal values.Such dynamics of the indicators of immune homeostasis in the skin indicates that the final completion of immune inflammation in the body and skin does not occur, and therefore the patient has a chronic and relapsed disease. Thus, the results of histological analysis of the skin at the atrophic stage of OS reflect the extinction of the immune process as sclerotic and atrophic changes in the skin develop, but none of the indicators tend to normalize, and this is due to the fact that in the sclerotic, and even more so in the atrophic the stage of LS is no longer the decay products in the amount that was at the erythematous stage. The general state of histological changes in LS can be characterized as activation of the T-system of immunity, inhibition of the B-system of immunity. High rates of CD3, CD4 and CD8 from the very beginning of the disease and their weak dynamics indicate that a high activity of immune inflammation damages the walls of blood vessels with the development of plasma leakage, fibrinoid swelling and fibrinoid necrosis surrounding tissue with collagen destruction.

A significant decrease in the levels of CD4, IL-1 and IL-2 producing cells, the number of CD16 macrophages and a decrease in the immunoregulatory index are a prognostic sign of the transition to the next stage.

It should also be noted that in patients with LS in the atrophic stage, there is no normalization of all immunological parameters characterizing the absence of remission, but only clinical improvement against the background of persistent disease.

Conclusion

Depending on the stage of the inflammatory process of LS, immune disorders are accompanied by a decrease in the total amount of blood lymphocytes due to subpopulations, namely CD4 T-helper cells and CD8 T-suppressors, CD54 endotheliocyte adhesion molecule, especially in the stages of erythema and edema or sclerosis. Due to the destruction of cellular contacts of endotheliocytes, the vesselwalls thickened, develop plasma impregnation and migration of immune cells into the perivascular space and dermis, which is confirmed by histological studies at different stages of the pathological process.

The study confirmed the important role of the activation of the T-cell link of the immune system in the pathogenesis of OS development, characterized by the appearance of immune inflammation, mainly from CD4 and CD8 lymphocytes, as well as producing cells of IL-1 and IL-2, which are inflammatory mediators. Interpretation of the results of immunograms in patients with LS revealed a significant increase in the blood of pro-inflammatory - IL-1β, IL-6, TNFα, anti-inflammatory (IL-4) cytokines and macrophages CD16, B-lymphocytes CD20, activator IL-2 CD25 with a simultaneous decrease in IFN-γ, which is evidence of the activation of both T and B-systems of immunity.

LS at all its stages, accompanied by an increase in blood levels of class G and M immunoglobulins, CIC. A decrease in the level of CD8 lymphocytes contributes to an increase in the level of CICs, which damage the basement membrane, and contribute to the acquisition of damaged antigenic characteristics and production of autoantibodies and new immune complexes. The blood level of markers of the early stage of cell activation, CD25, IL-1β, IL-4, IL-6, TNFα, is a diagnostic sign of inflammatory activity in children with LS. These indicators represent highly sensitive markers in assessing immunological changes, and are also informative for the determination of the course, prognosis and further treatment.

Study Limitations: The amount of participant in the control group was less than that of main group to compare. It might have affect some of our results. Thus, further researches can be conducted with same amount of participants in each arm.

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