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Effect of Azathioprine on Skin and Lung Parameters in Patients with Systemic Sclerosis: A Systematic Literature Review

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ABSTRACT

Introduction: Immunosuppressives can be effective in skin and lung involvement in systemic sclerosis(SSc). This study aims to analyse the efficacy of azathioprine in skin and lung parameters in SSc patients, comparing its efficacy with other immunosuppressives.

Method: A systematic literature review was performed. "Patient, intervention, comparison, outcome" method was used to screen studies. All studies that reported pretreatment and posttreatment modified Rodnan skin score (mRSS), forced vital capacity (FVC) and diffusşon capacity for carbon monoxide (DLCO) in SSc patients were included. Meta-analysis was performed where "mean difference" was used to assess the size of the change of pretreatment and posttreatment mean mRSS, FVC and DLCO values in azathioprine group and comparison group.

Results: Ten studies were included. One study demonstrated the role of azathioprine in maintaining the improvement in mRSS after induction treatment with cyclophosphamide. Five studies demonstrated the role of azathioprine as a maintenance agent in SSc interstitial lung disease after cyclophosphamide and three retrospective studies demonstrated the role of azathioprine alone in preserving FVC and DLCO. Two studies were included in meta-analysis of azathioprine and cyclophosphamide, which did not demonstrate significant differences between two drugs in terms of the change in mRSS, FVC and DLCO.

Conclusion: Azathioprine seems to have a role in stabilizing lung function tests after induction treatment with cyclophosphamide. Azathioprine alone can be effective in preserving lung functions a subset of SSc patients with interstitial lung disease. There is no evidence to use azathioprine alone in treatment of skin disease. The failure of meta-analysis to find significant difference between azathioprine and cyclophosphamide is probably due to the major differences in the characteristics of the study populations.

Key points: -There is no evidence that supports use of azathioprine as a monotherapy for skin involvement in systemic sclerosis. Although azathioprine alone may be beneficial in some systemic sclerosis patients with interstitial lung disease, most of the evidence supports its use as a maintenance agent after induction therapy with cyclophosphamide.

-Meta-analyses failed to demonstrate signficant difference between azathioprine and cyclophosphamide, but this is most likely due to the different characteristics of the patient populations in the included studies.

-This is the most extensive systematic literature review that was performed on the effects of azathioprine in skin and lung involvements of systemic sclerosis in the last two decades, which brings together all the recent available data on this drug.

Keywords: Azathioprine, lung, review, skin, systemic sclerosis.

Introduction

Azathioprine (AZA) is a purine antagonist immunosuppressive agent that inhibits leukocyte proliferation[1]. Azathioprine is effective as a maintenance agent after induction therapy with cyclophosphamide (CYC) in patients with lupus nephritis and systemic vasculitis[2]. In daily rheumatology practice, azathioprine has been largely replaced by mycophenolate mofetil (MMF) after the its successful use as a remission induction and maintenance agent in various autoimmune connective tissue diseases. Currently AZA is mainly used in special scenarios like pregnancy, where MMF is contraindicated[3].

Systemic sclerosis (SSc) is a disease characterized by autoimmunity, vasculopathy and fibrosis of skin and internal organs, with a significant disease burden and relatively higher mortality rate compared to other autoimmune connective tissue diseases[4]. Immunosuppressives can be effective in skin and lung involvement in SSc patients[5].

Various studies explored the efficacy of conventional immunosuppressives such cyclophosphamide and mycophenolate mofetil in systemic sclerosis patients especially in the setting of interstitial lung disease associated with systemic sclerosis[6,7]. Efficacy of these two agents has been subject of systematic literature reviews and meta-analyses[8,9]. In comparison to CYC and MMF, the efficacy of azathioprine has not been as extensively analyzed in systemic sclerosis. The evidence for the efficacy of AZA in skin and lung involvement of scleroderma patients may not be as robust as the evidence for the other two conventional immunosuppressive agents.

Therefore, this systematic review aims to accumulate all the available evidence on the use of azathioprine in systemic sclerosis by analyzing the efficacy of azathioprine on parameters that assess skin involvement (modified Rodnan skin score) and lung involvement (forced vital capacity and diffusion capacity for carbon monoxide) in patients with systemic sclerosis, comparing the efficacy of this agent with other immunosuppressives whenever the comparison is possible.

Method

International prospective register of systematic reviews (PROSPERO) ID number of this review is CRD42023415078. This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement for reporting of systematic reviews[10]. Since this study is a systematic literature review and meta-analysis, it was exempt from ethical approval.

The PICO method was used to screen studies. P-"patient": SSc patients older than 18 years that recieved immunosuppressive treatment, whose pretreatment and posttreatment mean modified Rodnan skin score (mRSS), forced vital capacity (FVC) and carbon monoxide (DLCO) values could be obtained, were included. I- "intervention": Azathioprine. C-"comparison": Other immunosuppressive agents. O-"outcome": Change in mRSS, FVC and diffusion capacity for DLCO after treatment with azathioprine or other immunosuppressive agents.

Studies published in languages other than English, case reports and studies that whose full text could not be obtained were excluded from the study.

All the studies that were published in English between 1 January 2000 and 31 December 2022 in Cochrane Database, Embase, MEDLINE, Ovid and Web of Science were searched. Keywords that were used were "scleroderma" or "systemic sclerosis" or "lung" or "interstitial lung disease" or "skin". Different types of studies were included with no limitation of study types as long as pretreatment and posttreatment mRSS, FVC and DLCO were present.

Two reviewers (EST and AB) independently identified the studies to be included by first reading the abstracts and excluding the duplicates and then by reading the full texts of the selected abstracts. From each of the selected studies they extracted the following information: First author, year of publication, study population characteristics, study design, pretreatment and posttreatment mRSS, FVC and DLCO values. For papers included

by only one reviewer a final decision was reached with the involvement of a third reviewer (BU).

Two authors (EST and EE) independently assessed the risk of bias (RoB) of each study. RoB was based on Cochrane Risk of Bias tool for randomized controlled trials and the Newcastle-Ottawa Scale for the cohort studies[11,12]. In case of disagreement, a final decision was reached by the discussion of the two reviewers.

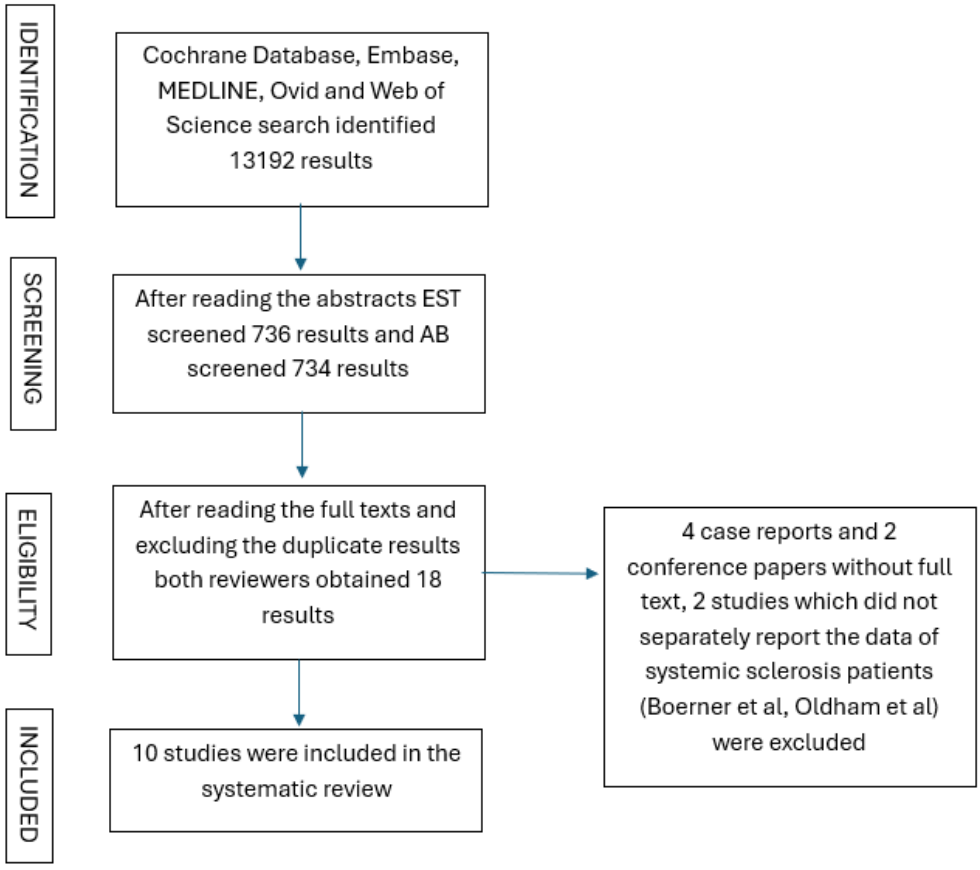
Begg and Mazumdar rank correlation test was used to test for publication bias in the studies that were included in the meta-analysis. Cochrane Q and I2 index were used to determine the heterogeneity of the studies. "Mean difference" was used as the effect size of the change of pretreatment and posttreatment mean mRSS, FVC and DLCO values in AZA group and comparison group. Meta-analyses of the mean values was reported under fixed effect and random effect models with weight coefficients and 95% confidence interval(CI). Forest plots of the meta-analyses was demonstrated by the magnitude of the weight of each study with 95% CI. MedCalc statistical software was used.

Quality of the evidence of the meta-analysis for each outcome (Change in mean mRSS, FVC and DLCO) were assessed using the GRADE Handbook[13].

Results

The flowchart is demontrated in Figure 1. After reading the full texts and removing the duplicate results both EST and AB obtained the same 18 results. Among these four case reports, two conference papers without a full text,and two studies were excluded because they didn't separately report the data of systemic sclerosis patients. Ten studies were used for the systematic review[14-23]. Brief descriptions of these studies are in Table 1.

Figure 1



Flowchart of the study

Table 1 Design and Brief Description of the Studies Selected for Systematic Review

Study	Study Design	Brief Description of the Study
Bérezné-2008[10]	Retrospective Multicenter Open-label Study	6 months of IV CYC followed by 18 months of AZA in 27 SSc patients with worsening ILD
Dheda-2004[11]	Retrospective Study	11 SSc-ILD patients treated with AZA, 3 patients priorly received CYC. 8 patients received at least 12 months of AZA
Hoyles- 2006[12]	Multicenter, Prospective, Randomized, Double-Blind, Placebo-Controlled Study	45 SSc patients with early pulmonary fibrosis, 22 patients received 6 months IV CYC followed by 6 months of AZA, 23 patients received placebo
Iudici- 2014[13]	Prospective, Observational Study	45 SSc patients with deteriorating ILD received IV pulse CYC, 39 completed CYC. 24 patients with improved or stable pulmonary function received AZA with a median follow up of 39 months, other 15 patients received MMF with a median follow up of 47 months
Kiboshi- 2021[14]	Retrospective Study	36 SSc-ILD patients, 18 patients received TAC, 18 received AZA, evaluated at 12th month and long period (>36 months)
Kundu- 2016[15]	Single-center, Prospective, Observational, Open-label Study	9 SSc-ILD patients received 6 months of IV CYC followed by 6 months of AZA, evaluated at 12 months
Nadashkevich- 2006[16]	Randomized Unblinded Trial	60 patients with early dcSSc- 30 received oral CYC, 30 received AZA for 18 months
Owen-2016[17]	Multicenter Prospective Cohort Study	47 SSc-ILD patients (36 patients priorly received CYC), 29 patients treated with AZA, 18 patients treated with MMF for 36 months
Paone- 2007[18]	Prospective Open-label Study	13 early dcSSc patients who received IV CYC for 12 months, then treated by AZA for 12 months
Poormoghim-2014[19]	Retrospective Cohort Study	36 SSc-ILD patients, 21 patients received oral CYC, 15 patients received AZA for 12 months

Abbreviations: AZA: azathioprine, CYC: cyclophosphamide, dcSSc: diffuse cutaneous systemic sclerosis, ILD: interstitial lung disease, IV: intravenous, MMF: mycophenolate mofetil, SSc: Systemic sclerosis; SSc-ILD: systemic sclerosis associated interstitial lung disease, TAC: tacrolimus

EFFECT OF AZATHIOPRINE ON MODIFIED RODNAN SKIN SCORE

Three studies reported pretreatment and posttreatment mRSS[20,22,23].

In the prospective open-labelled study by Paone, 13 early dcSSc patients received low-dose intravenous cyclophosphamide regimen for 1 year and then received azathioprine as a maintenance agent for 1 year. All patients also received low dose prednisolone. Mean mRSS significantly decreased (p=0.001) at the end of CYC treatment. Mean mRSS decreased further at the end of AZA treatment (p= 0.01). The decrease after AZA could be result of the late effect of CYC, rather than a direct effect of AZA itself. Therefore this study demonstrated the role of AZA as a maintenance agent in skin involvement after induction with CYC[22].

Study of Nadaskevich randomized 60 early diffuse cutaneous systemic sclerosis patients: Thirty patients received oral CYC and 30 patients received AZA.

Patients in both groups received prednisolone. Baseline mRSS of the groups were similar. At 18th month, CYC caused a significant reduction in mean mRSS ($p<0.001$). Unlike CYC, AZA failed to demonstrate a significant reduction in mRSS[20].

In the retrospective cohort study of 36 SSc patients with interstitial lung disease (SSc-ILD), Poormoghim compared 21 patients that recieved oral cyclophosphamide and 15 patients that received azathioprine. All patients receieved prednisolone. Changes of mRSS at AZA group and CYC groups at the end of 12th month were not significant. Despite neither drug causing a significant improvement, it should be noted that cyclophosphamide group had significantly higher initial mRSS scores compared to azathioprine group ($p=0.04$), which implies that CYC was utilized for more severe patients. Major limitations of this study were small size population and its retrospective nature, which inevitably made the study unblinded with selection biases[23].

EFFECT OF AZATHIOPRINE ON FORCED VITAL CAPACITY AND DIFFUSION CAPACITY FOR CARBON MONOXIDE

Studies Where Azathioprine is the Only Treatment

In the retrospective study by Dheda, 11 patients that had interstitial lung disease associated with systemic sclerosis received azathioprine and low dose prednisone. Three patients had to discontinue AZA due to adverse effects. The remianing 8 patients received AZA for 21.88 ±1.62 months. Three patients had priorly received cyclophosphamide but they all relapsed after CYC was terminated. Baseline FVC improved at 12 months and at 18 months, but these improvements were not statistically significant. This study demonstrates that AZA may have a role in stabilising lung function in a subset of SSc patients with interstital lung disease[15].

Studies Where Azatioprine is Given as a Maintenance Agent After Cyclophosphamide

Studies by Bérezné and Paone report the changes in FVC and DLCO before and after azathioprine treatment in SSc patients with interstitial lung disease that receive AZA immediately after finishing an induction regimen of cyclophosphamide[14,22]. Studies by Hoyles and Kundu report the changes in forced vital capacity and diffusion capacity for carbon monoxide before cyclophosphamide induction and after maintenance therapy with azathioprine[16,19]. Study by Iudici enrolled systemic sclerosis patients with lung involvement that had worsening lung function tests in the previous 6 months and reported the baseline FVC and DLCO of the patients. Patients that responded to CYC received maintenance treatment with azathioprine. At the end of the AZA treatment, the number of patients whose lung function tests improved, stabilized and worsened were reported[17].

In a retrospective, multicenter, open-label study by Bérezné, 27 scleroderma patients who had worsening interstitial lung disease were included. Patients received intravenous pulse cyclophosphamide per month for 6 months, and in case of stabilization or improvement azathioprine was prescribed as a maintenance treatment for 18 months. At the end of CYC treatment in the 6th month, in 7 patients the lung involvement improved, in 12 patients it was stabilized, and in 8 patients it worsened. Twenty-three patients completed two-year follow-up, 3 died, and one dropped out. Six had improved, 8 were stable, and 13 had worsened. Overall, CYC followed by maintenance with AZA was associated with stable or improved FVC in 70% and 51.8 % of patients at 6 months and 2 years, respectively. This study demonstrates that azathioprine may have a role in some SSc patients with lung involvement as a maintenance agent after cyclophosphamide treatment[14].

In a prospective open-label study by Paone, 13 systemic sclerosis patients with early diffuse cutaneous involvement received low-dose intravenous CYC regimen for 12 months, followed by azathioprine for 12 months. All patients also received low dose prednisolone . Baseline forced vital capcity and diffusion capacity for carbon monoxide increased at the end of CYC treatment ($p=0.001$ and 0.008 respectively). Improvements in

FVC and DLCO were maintained at the end of 12 months of AZA treatment. This study suggested a role for AZA in maintaining the improvement induced by cyclophosphamide. Authors raise the possibility that the results obtained at the end of the study could also be due to a "late effect" of cyclophosphamide[22].

In a multicenter, prospective, randomized, double-blind, placebo-controlled study by Hoyles , 45 SSc patients with early pulmonary fibrosis were randomized to receive treatment (oral prednisolone and 6 doses of monthly intravenous CYC followed by AZA, n=22) or placebo (n=23) for 1 year. Baseline FVC and DLCO of both arms were similar. At the end of 1 year, 19 patients in the treatment arm and 18 patients in the placebo arm completed the study. Forced vital capacity and diffusion capacity for carbon monoxide at the end of the study in the treatment arm was not significantly different from the same parameters in the placebo arm. This trial did not demonstrate significant improvement in neither parameter but there was a trend toward statistical significance in forced vital capacity (p=0.08). Authors stated that population selected for the study was characterized by mild functional impairment and, thus, only a minor therapeutic benefit was likely to be attainable. Limited sample size could have prevented the FVC change in the treatment arm from reaching statistical significance. In conclusion, treatment of interstitial lung disease in systemic sclerosis patients with low-dose prednisolone and intravenous cyclophosphamide followed by azathioprine stabilizes lung function in a subset of patients[16].

In their single-center, prospective, observational, open-label study Kundu enrolled 9 SSc patients with active alveolitis which was defined by the presence of any ground-glass opacity in computed tomography. FVC of the patients were between 30% and 85%. All the patients were treated with monthly intravenous pulse cyclophosphamide for 6 months, followed by azathioprine along with low-dose corticosteroids for one year. Baseline FVC of the patients significantly increased at the end of one year (p=0.003). Sequential treatment with intravenous CYC therapy for six months followed by AZA along with low-dose corticosteroids in these patients showed significant improvement of pulmonary function[19].

In a prospective, observational study, Iudici enrolled 45 SSc patients with worsening lung function in the previous 6 months. Patients were treated with weekly pulses of 500 mg of cyclophosphamide up to a cumulative dose of 10 g. All patients received low dose prednisone. At completion of the cyclophosphamide pulses, patients with improved or stable disease were defined as "CYC responders," who were treated with azathioprine. Patients were monitored for a median of 48 months. Six patients dropped out during the induction therapy. Among the remaining 39 patients, 24 improved or had stable pulmonary function tests. These 24 "CYC responders" were treated with AZA for a median of 39 months. In these patients 3 had improvement in forced vital capacity, 18 had stable FVC and 3 had worsened forced vital capacity and DLCO worsened only in 1 patient who had a parallel decline of FVC. These results support the use of low-dose pulse cyclophosphamide, followed by maintenance treatment with azathioprine in CYC responders in SSc patients with interstitial lung disease who had worsening lung function[17].

Azathioprine is Compared with Other Immunosuppressives

Comparison of Azathioprine and Cyclophosphamide

Study of Nadaskevich randomized 60 systemic sclerosis patients with early diffuse cutaneous involvement: Thirty patients received oral cyclophosphamide and 30 patients received azathioprine. Patients in both groups received prednisolone. Only one patient in both AZA and CYC groups had X-ray evidence for bibasilar pulmonary fibrosis. There was no significant difference between baseline forced vital capacity and diffusion capacity for carbon monoxide of the two groups. At the end of 18 months, there was a significant worsening in both FVC and DLCO in the

azathioprine group whereas these two parameters did not change significantly in cyclophosphamide group. At the completion of study, there were no additional patients with bibasilar pulmonary fibrosis in CYC-group, but in the AZA group signs of bibasilar pulmonary fibrosis were observed for the first time in five new patients. In this study cyclophoshamide was considered as a promising disease modifying medication for SSc, with positive effects on mRSS and a positive influence on the evolution of disease with a possible role in preventing of progression to pulmonary fibrosis[20].

In the retrospective cohort study of 36 SSc patients with interstitial lung disease, Poormoghim compared 21 patients that recieved oral CYC and 15 patients that received AZA. Baseline forced vital capacity and diffusion capacity for carbon monoxide of the two groups were not significantly different. At the end of 12 months, FVC and DLCO of the patients in azathioprine group significantly increased (p=0.05 and 0.01 respectively), whereas these parameters did not significantly change in cyclophosphamide group. However patients in the CYC group were younger at time of diagnosis of scleroderma associated interstitial lung disease, had more diffuse subtype, shorter duration of disease and less ground glass pattern on computed tomography. Two groups were not similar at their baseline presentation. Major limitations of this study were small size population and retrospective nature, which inevitably made the study unblinded with selection biases. However results demonstrate that azathioprine had beneficial effects in some systemic sclerosis patients with interstitial lung disease[23].

Comparison of Azathioprine and Mycophenolate Mofetil

In a multicenter prospective cohort study by Owen, 29 patients treated with azathioprine and 18 patients treated with mycophanolate mofetil were analysed. There was no significant difference between the groups with respect to duration of systemic sclerosis and duration of lung involvement. Baseline median forced vital capacity was higher in the AZA group with a trend towards statistical significance (p=0.06). Baseline median diffusion capacity for carbon monoxide were similar in two groups. Fourteen patients in the MMF group and 22 patients in the AZA group had received prior cyclophosphamide. Seventeen patients in the mycophenolate group and 24 patients in the azathioprine group were concurrently treated with prednisolone. In the AZA group, median absolute FVC and DLCO of the patients did not significantly decline in the year prior to azathioprine initiation. However the decline in diffusion capacity for carbon monoxide trended toward significance (p=0.07). After initiation of azathioprine, both parameters continued to remain stable at 12th, 24th and 36th months of treatment. In MMF group median absolute FVC of the patients significantly declined in the year prior to mycophenolate initiation(p=0.02). Decline of median absolute DLCO of the patients trended towards significance in the year prior to MMF initiation (p=0.07). After initiation of mycophenolate mofetil, both parameters stabilized at 12th, 24th and 36th months of treatment[21].

In this study, patients treated with azathioprine seemed to have less progressive disease than those who received mycophenolate mofetil. In contrast to MMF where stability was demonstrated in progressive interstitial lung disease, conclusions regarding the efficacy of azathioprine in scleroderma patients with interstitial lung disease that had declining pulmonary function cannot be drawn from the study. A possible limitation of the study was the prior cyclophosphamide use in a majority of the patients, which made it impossible to be certain that the stability in these respiratory function test parameters is attributable to MMF and AZA alone[21].

Comparison of Azathioprine and Tacrolimus

A retrospective study by Kiboshi compared the efficacy of azathioprine and tacrolimus in systemic sclerosis patients with interstitial lung disease. Eighteen patients that received a combination of

AZA and prednisolone were compared with 18 patients that received a combination of tacrolimus and prednisolone. There was no significant difference between baseline forced vital capacity and diffusion capacity for carbon monoxide values of the two groups but forced vital capacity was lower in azathioprine group that trended toward statistical significance (p=0.052). Baseline FVC and DLCO of azathioprine group did not change significantly at 12th month and at the endpoint (median treatment duration: 98 months). At 12th month of the study 6 patients improved and 12 patients were stable and at the endpoint 2 patients improved, 9 patients were stable, only one patient worsened. Baseline FVC and DLCO of tacrolimus group didn't change significantly at 12th month and at the endpoint (median treatment duration: 65.8 months). At 12th month of the study 5 patients improved and 13 patients were stable and at the endpoint 4 patients improved, 7 patients were stable. Prednisolone dose at 12th month and at the endpoint were both significantly lower than the prednizolone dose of the baseline in both groups. The limitations of this study were the small number of patients, its retrospective and single-center nature. In conclusion, combination therapies with both prednisolone and azathioprine or tacrolimus were effective in systemic sclerosis patients with interstitial lung disease[18].

META-ANALYSES

Only the studies by Nadashkevich and Poormoghim provided head to head comparison of AZA with CYC. Both studies provided mRSS, FVC and DLCO for both azathioprine and cyclophosphamide treatment groups at the beginning and at 12th month of treatment[20,23].

The risk of bias of the randomized controlled study by Nadashkevich[20] was analyzed according to Cochrane Risk of Bias tool for randomized controlled trials[11]. This study had a high risk of bias. The risk of bias of the retrospective cohort study by Poormoghim[23] was assessed according to Newcastle-Ottawa Scale[12]. This study had a score of 7, therefore a low risk of bias. These results are demonstrated in Supplementary Table 1 and Supplementary Table 2.

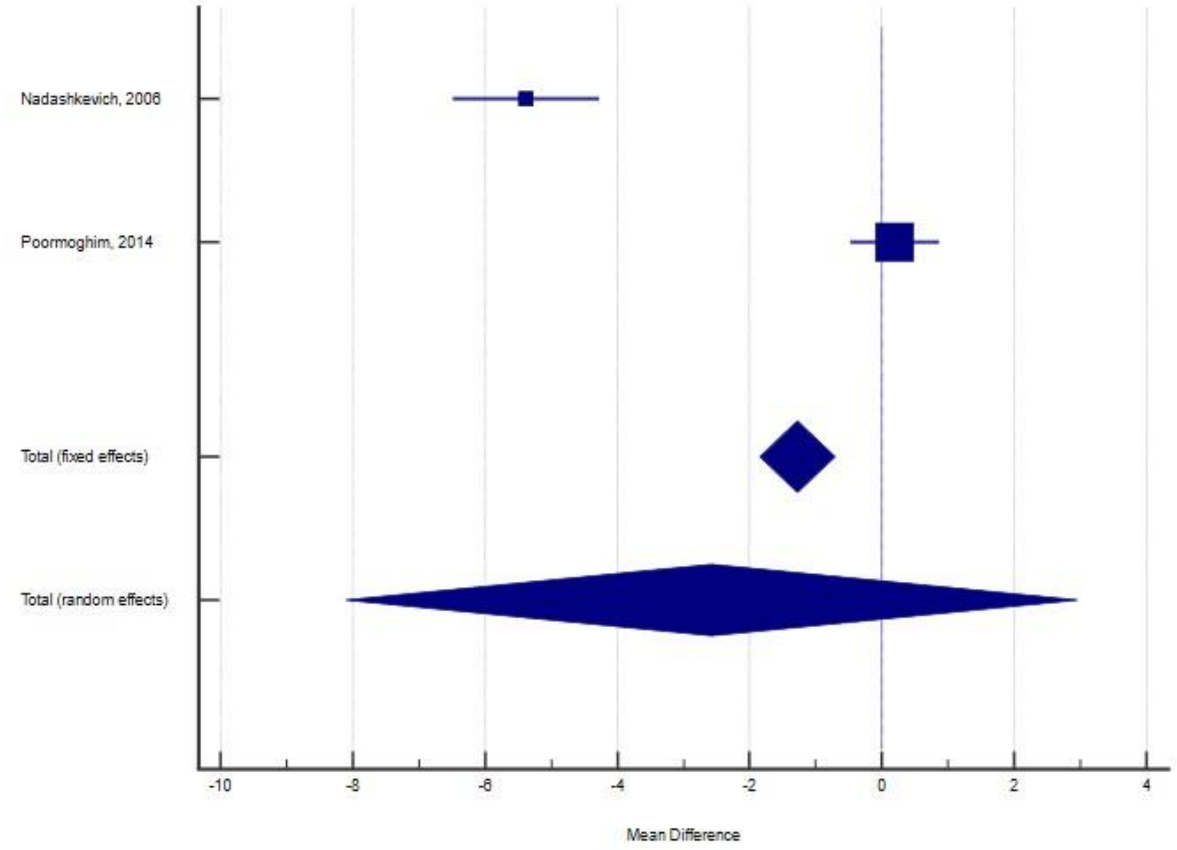
Study by Nadashkevich enrolled 30 patients in the azathioprine group (Twenty-seven female, 3 male; median age 36) and 30 patients in the cyclophosphamide group (twenty-six female, 4 male; median age 38)[20]. Study by Poormoghim included 15 patients in the AZA group (Twelve female, 3 male; median age 42) and 21 patients in the CYC group (Eighteen female, 3 male; median age 34)[23].

For modified Rodnan skin score, forced vital capacity and diffusion capacity for carbon monoxide parameters, publication bias was assessed separately. Kendall's Tau was 0.99 and p value was >0.05 for all three parameters, therefore there was no publication bias.

For mRSS, FVC and DLCO parameters publication bias was assessed separately. I2 was 98.66 for mRSS, 98.38 for FVC and 98.62 for DLCO with p<0.001 for all three. For all three parameters there was a high level of heterogeneity and therefore random effect model was used for the meta-analyses.

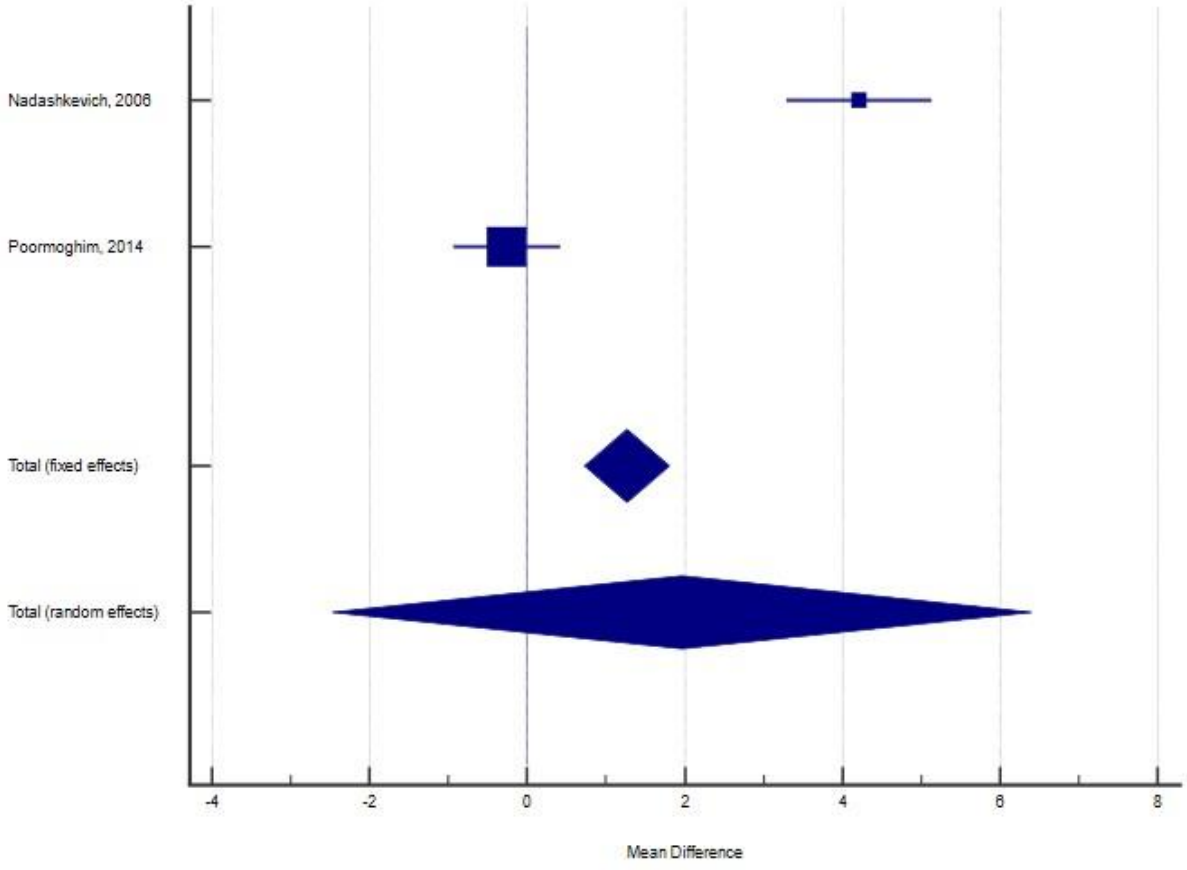
Mean difference of modified Rodnan skin score is -2.57 (-8.11, 2.96) (p=0.36). Mean difference of forced vital capacity is 1.96 (-2.47, 6.4) (p=0.38). Mean difference of diffusion capacity for carbon monoxide is 1.71 (-3.11, 6.51) (p=0.48). Therefore there was no significant difference between azathioprine and cyclophosphamide groups in terms of the change in mean mRSS, FVC and DLCO. Forest plots for modified Rodnan skin score, forced vital capacity and diffusion capacity for carbon monoxide are demonstrated in Figures 2,3 and 4 respectively.

Figure 2



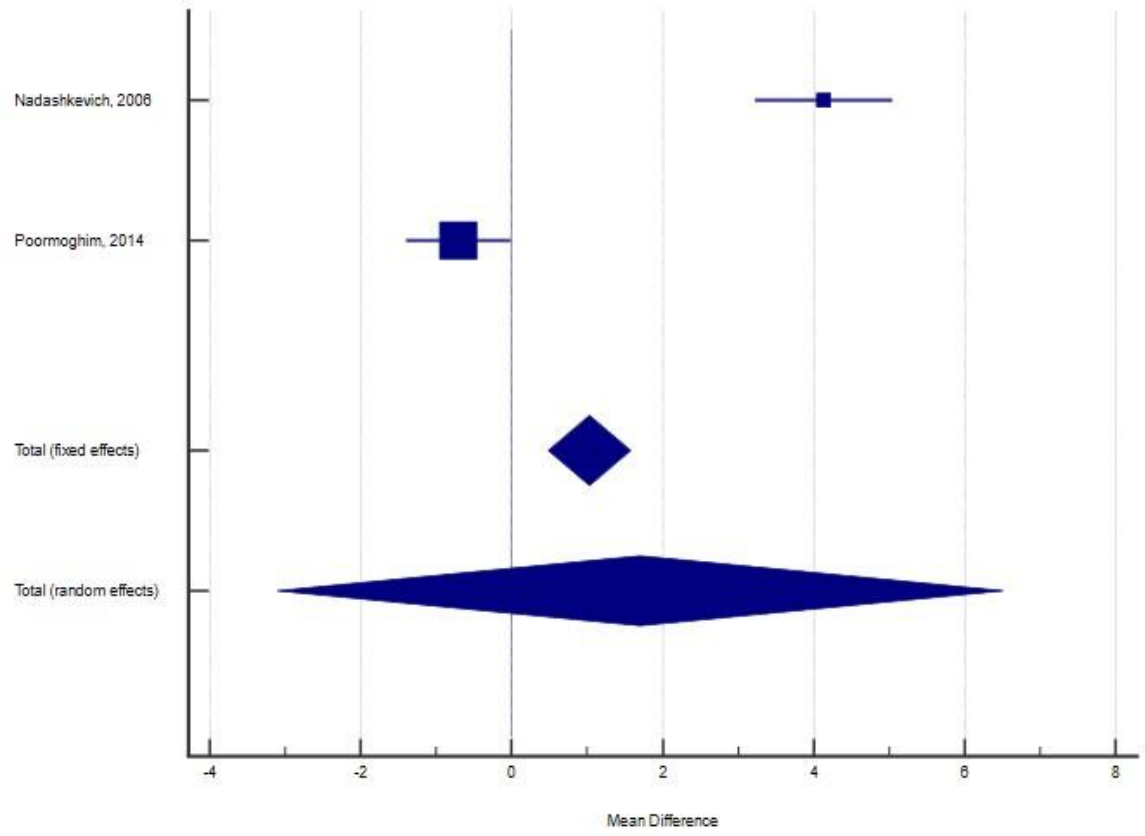
Forest plot of the mean difference of modified Rodnan skin score in azathioprine and cyclophosphamide groups

Figure 3



Forest plot of the mean difference of forced vital capacty in azathioprine and cyclophosphamide groups

Figure 4



Forest plot of the mean difference of diffusion capacity for carbon monoxide in azathioprine and cyclophosphamide groups

Quality assessment of the meta-analyses for mRSS, FVC and DLCO according to GRADE Handbook. For all the three parameters the starting grade was "low", because the observational study of Poormoghim was included in the meta-analyses[23]. Due to high risk of bias from randomized study of Nadashkevich, inconsistency of results (high heterogeneity) and imprecision, the evidence obtained from the meta-analyses of each parameter yielded "very low quality" of evidence[20,23].

Discussion

Mycophenolate mofetil and cyclophosphamide are potent conventional immunosuppressive agents whose efficacy in systemic sclerosis has been explored in previous studies and reviews[6,7,8,9]. In comparison to these two agents, azathioprine's efficacy in systemic sclerosis has not been as thoroughly analyzed, which was the main purpose of this review. This study is the most extensive systematic literature review that was performed on the efficacy of azathioprine in skin and lung involvements of systemic sclerosis in the last two decades.

In the study by Paone, after induction with cyclophosphamide, azathioprine maintained the improvement in modifid Rodnan skin score and even caused a further decrease in mRSS. Azathioprine may have a role as a maintenance agent in skin involvement after induction with cyclophosphamide[20]. In the study by Nadaskevich in 30 systemic sclerosis patients with early diffuse skin involvement and in the study by Poormoghim in 15 systemic sclerosis patients among which only one had diffuse skin involvement, azathioprine failed to demonstrate a significant improvement in mRSS[20,23].

Thus, is no robust evidence for the use of azathioprine alone in order to improve modified Rodnan skin score in systemic sclerosis patients. It may only have a role in maintaining the improvement in this parameter, if it is used after cyclophosphamide. In this setting, the "late effect" of cyclophosphamide may also contribute to azathioprine's efficacy.

The studies that assessed the efficacy of azathioprine in improving forced vital capacity and diffusion capacity for carbon monoxide

demonstrated varying results. Five studies presented evidence that azathioprine has a role as a maintenance agent that stabilizes FVC and DLCO after induction treatment with cyclophosphamide[14,16,17,19,22]. When used in this setting, late effect of CYC may also contribute to the "stabilizing effect" of AZA. In a group of systemic sclerosis patients with early diffuse cutaneous involvement that have a high risk of progression to interstitial lung disease, azathioprine could not prevent the decline of respiratory function tests and the emergence of new cases of bibasilar pulmonary fibrosis[20]. In Owen's study, azathioprine group had relatively higher baseline forced vital capacity compared to mycophenolate mofetil group and FVC did not undergo a significant decline in the year prior to treatment. Therefore authors claimed that conclusions regarding the efficacy of azathioprine in scleroderma patients with declining pulmonary function tests could not be drawn[21]. In retrospective studies of Dheda, Poormoghim and Kiboshi, azathioprine alone may have a role in stabilizing respiratory function tests in some systemic sclerosis patients with interstitial lung disease[15,18,23].

Meta analyses of the studies by Nadashkevich and Poormoghim failed to demonstrate a significant difference between azathioprine and cyclophosphamide in terms of the change in modified Rodnan skin score, forced vital capacity and diffusion capacity for carbon monoxide. This result seems to differ from the observations in the daily clinical practice where the clinicians usually consider cyclophosphamide more "potent" than azathioprine and use azathiprine as a maintenance agent after induction treatment with cyclophosphamide.

However, there are certain limitations that should be considered while interpreting these results. First of all, the meta-analyses included two studies with different designs: the randomized unblinded trial by Nadashkevich[20] and the retrospective cohort study by Poormoghim[23].

Second and the most important factor is the different patient characteristics in these studies. Although mean mRSS of both patient groups were similar in study of Nadashkevich[20], in the study of Poormoghim only 1 patient in azathioprine group had diffuse skin involvement whereas 8 patients in the cyclophosphamide group had diffuse skin involvement. Initial modified Rodnan skin score of AZA group was significantly lower than that of CYC group ($p=0.04$)[23]. These significant differences in baseline characteristics most likely affected the outcome of the meta-analysis of mRSS.

In the study of Nadashkevich, only one patient in each azathioprine and cyclophosphamide groups had bibasilar pulmonary fibrosis. Baseline FVC and DLCO of both groups were similar. Therefore this study wasn't performed among systemic sclerosis patients with interstitial lung disease, but among systemic sclerosis patients with early diffuse skin involvement who were at "high risk" to develop interstitial lung disease[20]. However, the study by Poormoghim included 36 scleroderma patients with interstitial lung disease that had less than 70 percent of predicted forced vital capacity. Baseline forced vital capacity and diffusion capacity for carbon monoxide of AZA and CYC groups were not significantly different but some baseline characteristics differed significantly among two groups: patients in the cyclophosphamide group were were younger at time of diagnosis of interstitial lung disease, had more diffuse subtype, shorter duration of disease and less ground glass pattern on computed tomography[23]. This brings even more heterogeneity to the meta-analyses of FVC and DLCO. Thirdly, AZA, CYC and prednisolone doses differed between the studies[20,23]. Due to all of these factors, according to GRADE scoring system the qualities of the meta-analyses were "very low".

Limitations of this systematic literature review include a paucity of large scale randomized controlled studies in systemic sclerosis patients, its focus on modified Rodnan skin score, forced vital capacity, and diffusion capacity for carbon monoxide which quantify disease activity but may not always reflect clinically significant outcomes or

parameters related to patients' quality of life. Another limitation is the focus of this study on efficacy alone, not including the safety parameters related to azathioprine. Due to the limitations of the included studies our meta-analyses yielded very low quality of evidence.

With the widespread use of mycophenolate in systemic sclerosis skin involvement and both as an induction and maintenance agent in interstitial lung disease associated with systemic sclerosis[24], widespread use of rituximab in scleroderma interstitial lung disease [25], emerging use of tocilizumab for pulmonary involvement of systemic sclerosis[26] and inclusion of antifibrotic agent nintedanib into the therapeutic arsenal of scleroderma lung disease[27], it is highly unlikely that future studies on skin and lung involvement in SSc will focus on AZA. Therefore azathioprine will likely remain as a maintenance agent after cyclophosphamide in settings where mycophenolate mofetil is unavailable, is not tolerated or contraindicated in systemic sclerosis patients.

Conclusion

Thus, this systematic literature review and meta-analysis failed to find strong evidence for the use of azathioprine in order to improve modified Rodnan skin score. Azathioprine may only have a role in maintaining the improvement in mRSS if it is used after cyclophosphamide as a maintenance agent. Even in this context, improvement in the skin parameters during AZA use could be a result of the "late effect" of CYC. Evidence that azathioprine in combination with steroids could stabilize forced vital capacity and diffusion capacity for carbon monoxide in a subset of scleroderma patients with interstitial lung disease comes from three retrospective studies. Five studies demonstrate that AZA can have a role as a maintenance agent that stabilizes FVC and DLCO after induction treatment with cyclophosphamide. In this context, "late effect" of CYC may contribute to the stabilizing effect azathioprine.

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The authors declare no conflict of interest.

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EST, AB and BU took part in the data extraction process as described in the main text above and performed the systematic literature review. EE and EST performed the risk of bias analysis and quality analysis of the studies included in the meta-analyses. EE performed the meta-analyses. EST wrote the manuscript. EE, AB and BU made the necessary corrections and approved the final version.

Ethical Approval:

Ethical approval was not sought due to the nature of the study.

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Supplementary Table 1: Risk of Bias analysis of the study by Nadashkevich according to Cochrane Risk of Bias tool for randomized studies

Study	Nadashkevich et al.
Domain 1-Randomization process	High risk
1.1 Was the allocation sequence random?	1.1 No
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	1.2 No information
1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?	1.3 Probably yes
Domain 2-Deviations from intended interventions	Some concern
2.1 Were participants aware of their assigned intervention during the trial?	2.1 Yes
2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	2.2 Yes
2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context	2.3 No
2.4 If Y/PY to 2.3: Were these interventions likely to have affected the outcome?	2.4 Not available
2.5 If N/PN/NI to 2.4: Were these deviations from intended intervention balanced between groups?	2.5 Not available
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	2.6 No
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	2.7 No
Domain 3- Missing outcome data	Low risk
3.1 Were data for this outcome available for all, or nearly all, participants randomized?	3.1 Yes
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	3.2 Not available
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	3.3 Not available
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	3.4 Not available
Domain 4- Measurement of the outcome	Low risk
4.1 Was the method of measuring the outcome inappropriate?	4.1 No
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	4.2 No
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	4.3 Yes
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	4.4 Probably no
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	4.5 Not available
Domain 5 Selection of the reported result	Low risk
5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	5.1 Probably yes
5.2… multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	5.2 No
5.3… multiple eligible analyses of the data?	5.3 No
6. Other bias	6 No
Overall bias	High risk

Supplementary Table 2 Risk of Bias analysis of the study by Poormoghim according to Newcastle-Ottawa Scale for cohort studies

Study	Poormoghim et al.
1.Representativeness of the intervention cohort	
a)truly representative of the average systemic sclerosis patients *	☒
b)somewhat representative of the average systemic sclerosis patients *	☐
c)selected group of patients	☐
d)no description of the derivation of the cohort	☐
2.Selection of the non intervention cohort	
a)drawn from the same community as the intervention cohort *	☒
b)drawn from a different source	☐
c)no description of the derivation of the non intervention cohort	☐
3.Ascertainment of intervention	
a)secure record (eg health care record) *	☐
b)structured interview *	☐
c)written self report	☐
d)other / no description	☒
4.Demonstration that outcome of interest was not present at start of study	
a)yes *	☒
b)no	☐
Comparability	
1.Comparability of cohorts on the basis of the design or analysis	
a)study controls for limited or diffuse skin involvement, duration to develop ILD, HRCT pattern of ILD *	☐
b)study controls for any additional factors (age, sex, anti-topoisomerase positivity *	☒
Outcome	
1.Assessment of outcome	
a)independent blind assessment *	☒
b)record linkage *	☐
c)self report	☐
d)other / no description	☐
2.Was follow up long enough for outcomes to occur	
a)yes, if median duration of follow-up >= 6 month*	☒
b)no, if median duration of follow-up < 6 months	☐
3.Adequacy of follow up of cohorts	
a)complete follow up: all subjects accounted for *	☒
b)subjects lost to follow up unlikely to introduce bias: number lost <= 20%, or description of those lost suggesting no different from those followed*	☐
c)follow up rate < 80% (select an adequate %) and no description of those lost	☐
d)no statement	☐
Total points	7

Abbreviations: HRCT-high resolution computed tomography, ILD-interstitial lung disease