RESEARCH ARTICLE

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Allergic Reactions to *E. coli* Asparaginase are Associated with Decreased Asparaginase Activity in an Indonesian Pediatric Population with ALL

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Abstract

Purpose: The asparaginase's (ASP) utility for ALL treatment is limited by neutralizing antibodies, which is problematic in countries whose access limited to alternative preparations. ASP antibody levels and activity was measured during remission induction and associated with allergy manifestations. **Methods:** *E. coli* ASP was dosed at 7500 IU/m^2. ASP IgG antibody levels were quantified at the beginning and end of induction. ASP activity was measured 24 hours after 1st and 5th dose (standard-risk) or 7th dose (high-risk patients) administration, and within 24 hours in case of allergic reactions. Allergy was monitored by CTCAE version 3. Parametric and non-parametric was performed for data analysis. **Results:**ASP antibody and activity levels were available in 41/63 consecutive patients. Allergic manifestations occurred in 13/41, with urticaria being the most frequent. There were no significant differences in subject characteristics based on allergic reactions. The 5th dose was the most frequent time of onset. Antibody levels in allergy group at the end of induction did not differ from those at baseline ($p<0.05$). Using a 24-hour level of 100 mU/mL as a threshold for adequate ASP activity, 6/13 patients with allergy had adequate levels compared to 26/28 patients without ($p<0.05$). The ASP activity level at the end of induction phase in both groups did not show a significant decrement. **Conclusion:** The *E. coli* ASP activity with adequate levels were significantly higher in non-allergy group. Its activity level was not accompanied by increment of IgG in allergic group indicates other factors might affect activity levels in allergy group.

Keywords: Asparaginase activity- acute lymphoblastic leukemia- allergy- hypersensitivity, neutralization antibody

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Introduction

Serious drug-induced toxicity influences the failure of cancer therapy in developing countries and is closely related to the incidence of treatment-related mortality (TRM)(Howard and Wilimas, 2005; Gupta et al., 2011). TRM rates of ALL therapies is less than 5% in high-income countries, but can be 5–10 times higher in developing countries (Hunger and Antillon, 2014). Studies conducted in El Salvador, Honduras, and Guatemala showed that most therapy-related deaths (59%) occurred in the induction phase (Gupta et al., 2011).

Asparaginase is a cytostatic drug mainly used in the induction phase, apart from prednisone or dexamethasone, daunorubicin, and vincristine, and intrathecal medication (Gupta et al., 2011; Hunger and Antillon, 2014; Cooper and Brown, 2015; Roy et al., 2017). Asparaginase has been an important component of ALL treatment and since its first use in the 1960s, has contributed to the improved survival of pediatric ALL patients to now 90% (Egler et al., 2016). Asparaginase is an enzyme produced by bacteria that catalyzes the conversion of asparagine to aspartic acid and ammonia(Chabner et al., 2014; Asselin and Rizzari, 2015; Santos et al., 2017; Browne et al., 2018). It causes depletion of extracellular asparagine, an amino acid that cannot be synthesized by leukemia cells and that acts on almost the entire cell cycle (Pagliardi et al., 1973; Ueno et al., 1997). There are three formulas with different pharmacokinetics. The first formula is derived from *Escherichia coli* (ASP *E. coli*), the second

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is the natural pegylated form of *E. coli* (PEG-ASP), and the third is isolated from Erwinia chrysanthemi (crisantaspase or Erwinase) (Chabner et al., 2014; Inaba et al., 2015; Hijiya and Sluis, 2016; Santos et al., 2017; Browne et al., 2018). Currently, only *E. coli* ASP is available in Indonesia(Society, 2013). However, the use of ASP *E. coli* is associated with the risk of undesirable drug reactions, which are classified as immunological and non-immunological (Jaffe et al., 1971; Inaba et al., 2015; Hijiya and Sluis, 2016), Immunological reactions consist of allergic reactions with or without clinical manifestations (Asselin and Rizzari, 2015; Santos et al., 2017). which range from skin rashes, urticaria, itching, edema, bronchospasm to anaphylaxis(Jaffe et al., 1971; Avramis and Tiwari, 2006; Asselin and Rizzari, 2015; Chu et al., 2015; Browne et al., 2018). Allergic reactions are the most common, occurring in 30–45% of ALL patients(Hijiya and Sluis, 2016; Lanvers-Kaminsky, 2017), however, the reported incidence varies depending on the type, route of administration, and the dose of ASP (Lanvers-Kaminsky, 2017).

Most studies assessing the properties of anti-L-ASP antibodies evaluate IgG immunoglobulins and the role of IgM and Ig E antibodies in allergic reactions to L-ASP and inactivation of L-ASP remains unclear(Ueno et al., 1997; Asselin and Rizzari, 2015). A significant proportion of immune reactions against L-ASP occurs during the first minutes of drug administration, suggesting the significant involvement of mast cell degranulation, as well as the release of histamine and other inflammatory mediators, indicating that this process is Ig E-dependent. Furthermore, clinically drug overt allergy was typically associated with the presence of Ig E-class anti-drug antibodies (ADA), whereas enzyme inactivation mainly depends on specific IgG antibodies(Pagliardi et al., 1973; Inaba et al., 2015). This study aimed to determine the level of IgG anti-L-ASP antibodies during the induction and their association with L-ASP activity and allergic reactions to ASP. ASP efficacy can be quantified by measuring asparagine levels, ASP activity, and anti-asparaginase antibodies, with asparaginase activity and antibody measurements used as a basis for identifying patients with silent inactivation. Therapy ideally is modified in such patients (Salzer et al., 2018). To the best of the authors knowledge, research related to this topic has not been conducted in Indonesia. The study findings could be used for future recommendations on use of L-ASP and a state-of-the art ALL chemotherapy protocol including L-ASP.

Materials and Methods

Study Group

Newly diagnosed pediatric patients (1–18 years old) who were given 8 doses for high-risk group and 6 doses for standard risk group of ASP *E. coli* infusion during the induction phase of ALL treatment were recruited from the Chemotherapy Ward, Dr. Hasan Sadikin Hospital, Bandung from June 2019 until June 2020 as shown in Figure 1 without history of allergy or atopy.

Drug Administration

Each patient was given asparaginase infusion according to the National Protocol of ALL 2018 with ASP *E. coli* given 8 times in the 3rd to 6th weeks for high risk and 6 times in the 4th to 6th weeks for standard risk (Pagliardi et al., 1973). Asparaginase was given with 48-hour interval between administrations according to protocol 2018 (Asselin and Rizzari, 2015), 7,500 units/m^2 doses given intravenously in 100 mL of fluid for 1–2 hours. Patients who met the inclusion criteria underwent the research procedure detailed in the flow chart, see Figure 1

Operational Definition

L-ASP E. coli activity

L-ASP *E. coli* activity was measured 24 hours after administration of the 1st dose, the 5th dose in the standard risk patients, and the 7th dose for the high-risk group using the Abnova Asparaginase Activity Assay Kit, a simple, direct, and automation-ready procedure to colorimetrically measure asparaginase activity. For patients who experienced an allergic reaction, an additional measurement was performed 24 hours after the ASP dosing which caused the allergic manifestation. The patient samples were centrifuged at 10,000 RPM for 5 minutes, then 2–10 µL of the clarified sample and 5 µL of the asparaginase positive control were added to the 96-well plate and the volume adjusted to 50 µL/well using asparaginase assay buffer. The absorbance at OD570 was immediately measured in kinetic mode at 25°C for 30–60 minutes and a standard curve of aspartate generation was plotted. The ASP activity level was calculated according to the formula in the Abnova Manual and classified in the group with adequate and inadequate activity level. The activity level was considered adequate when the mean value of all measurements in each phase of treatment exceeded 100 mIU/L.

L-ASP E. coli antibodies

IgG anti-ASP *E. coli* antibody levels were measured before the 1st week of chemotherapy, together with bone marrow morphology evaluation in the 7th week, and within 24 hours of allergic manifestations in the allergic reaction group by ELISA (Mybiosource, China). IgG ELISA quantitation kits were purchased from Mybiosource (Affymetrix, San Diego, CA, USA). The sensitivity of this kit is 0.1 ng/mL, with a detection range of 0.625–20 ng/mL. Serum samples were obtained by centrifugation at 3000 rpm for approximately 20 minutes and stored at-80°C for not more than 6 months. Briefly, 50 µL of standards, blank or diluted serum samples were added to 96-well plates precoated with each specific antigen, then 100 µL of HRP conjugate was added to each well except the blank wells. After incubation for 1 h, 50 µL of chromogen A and B was added to all wells, mixed gently, and incubated for 15 minutes at 37°C. Finally, 50 µL of stop solution was added and the absorbance (450 nm) was measured in 5 minutes.

Allergic Manifestation

Allergic manifestations of L-ASP were defined according to the Common Terminology Criteria for

Adverse Events (CTCAE vol. 4.03) (Division and D, 2006).

Statistical Analysis

Continuous variables were summarized as median, range, mean, and standard deviation, while categorical variables were presented as a number. Variables were compared using unpaired t-test for parametric test and non-parametric tests such as Chi-square, Fisher exact, bivariate Mann-Whitney, and ANOVA. In this analysis, one observation for each patient was considered. Outcome events were allergic manifestations, ASP activity, and anti-ASP (IgG) antibodies measured at the end of the induction phase. The findings were considered statistically significant at p-values of <0.05 (2-sided).

Results

Of the initial 63 newly diagnosed patients, 41 were completely analyzed. There were no significant differences between patients based on presence or absence of allergic reactions (p>0.05, Table 1). Allergic reactions occurred in 13/41 patients (Table 2) with 19 events observed during study, urticaria (CTCAE grade 2) being the most frequent manifestation. Severity was grade 2 and 3 in 9/19 and 10/19 events, respectively. The 5th dose was the most frequent onset. All patients who experienced allergic reactions subsequently received the drug via the intramuscular route with the administration of diphenhydramine and dexamethasone premedication, except for one subject in the standard-risk group who experienced allergies at the 6th dose (last dose). After administration of the intramuscular dose, there was no reoccurrence of allergies in all patients and no patient underwent ASP desensitization procedure.

Using a 24-hour level of 100 mIU/mL as a threshold for adequate ASP activity, 6/13 patients with allergy had adequate levels, compared to 26/28 patients without allergy (p=0.001, Table 1). There was no antibody increment during repeated ASP administrations in induction phase. (Tables 1, 2 and Figure 2).

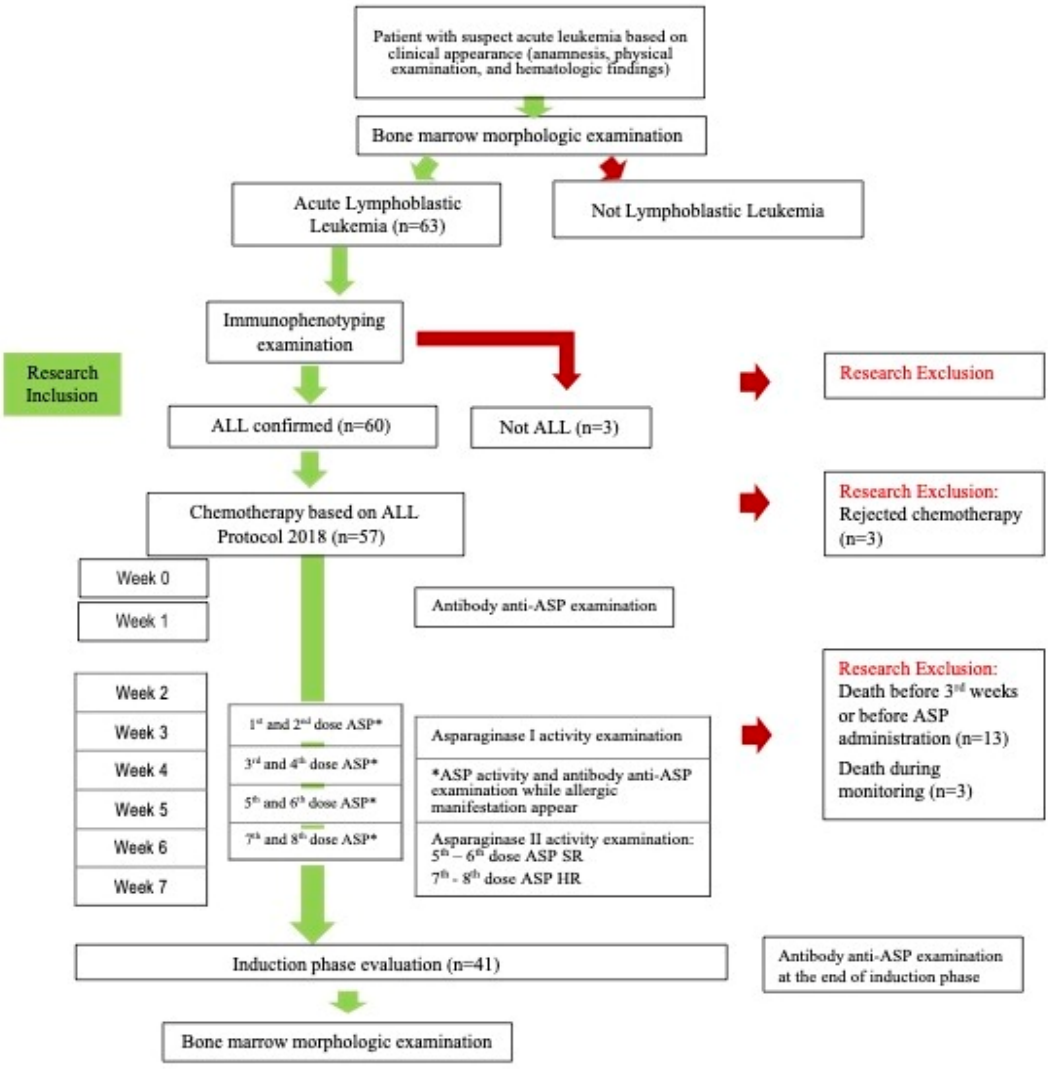

Figure 1. Research Protocol

Table 1. Study Group Characteristics, Antibody IgG and activity ASP *E. coli*

General Characteristic	Allergy n=13	Non-Allergy n=28	p
Age, median (range)	4 (1–16)	5 (1–16)	0.284[a]
Sex			
Male	8	18	0.566[b]
Female	5	10	
Nutrition status			
Malnutrition	10	15	0.139[b]
Not Malnutrition	3	13	
Risk Stratification			
Standard Risk	6	15	0.457[b]
High Risk	7	13	
Highest Leucocyte Levels			
<50.000	8	19	0.477[b]
>50.000	5	9	
Bone Marrow Morphology			
L1	1	2	0.773[b]
L2	10	2	
Undetermined	2	2	
munophenotyping			N/A
B cell	10	24	
T cell	0	2	
Aberrans	3	1	
Undetermined	0	1	
Bone Marrow Evaluation			
Complete remission	12	27	
Partial and no remission	1	1	0.539[b]
Induction phase duration (days)			
Median (min–max.);			
Mean±SD	78 (64–118); 83,54+16,11	73 (52–141); 78,71+21,47)	0.402[a]
Antibody E. coli ASP (ng/mL) median (range)	Allergy	Non-Allergy	p
	n=13	n=28	
Initial	3.88+3.83	2.99+2.75	>0.05[c]
Onset of Allergy	4.49+3.55	NA	
End of Induction	4.67+3.38	7.16 (0.24–19.08)	>0.05[d]
Activity *E. coli* ASP End of induction	Allergy	Non-Allergy	P
(Level Adequacy)*	n=13	n=28	
Adequate	6	26	<0.05[e]
Non adequate	7	2	

[a], calculated by Chi-square test; [b], calculated by Fisher exact test; [c], calculated by independent T-test; [d], calculated by Mann-Whitney; [e], calculated by Mc Nemar; * 100 mIU/mL as a threshold for adequate ASP activity

Discussion

The immunological side-effects of ASP can be classified as type 1 hypersensitivity reactions (allergies) and the formation of neutralizing antibodies which can reduce ASP activity (Woo et al., 2000; Hijiya and Sluis, 2016; Sluis et al., 2016). The allergy frequency in this study was 32.4% in line with previous RSHS data for 2018–2019, with a reported allergy frequency of 37.6% (Sari et al., 2020). Other studies using different protocols reported that the incidence of ASP allergy varies widely, ranging between 10–60% (Panosyan et al., 2004; Liu et al., 2012) due to differences in formulation, route, and dose of administration (Szewczyk et al., 2004).

In this study, in 13 patients who experienced allergic manifestations, the most frequent symptoms were urticaria (Browne et al., 2018) followed by angioedema (Santos et al., 2017), and bronchospasm (Hunger and Antillon, 2014). It is of note that a patient could experience two manifestations at once. These findings are consistent with previous studies in RSHS which found urticaria to be the most common manifestation (Sari et al., 2020). The most

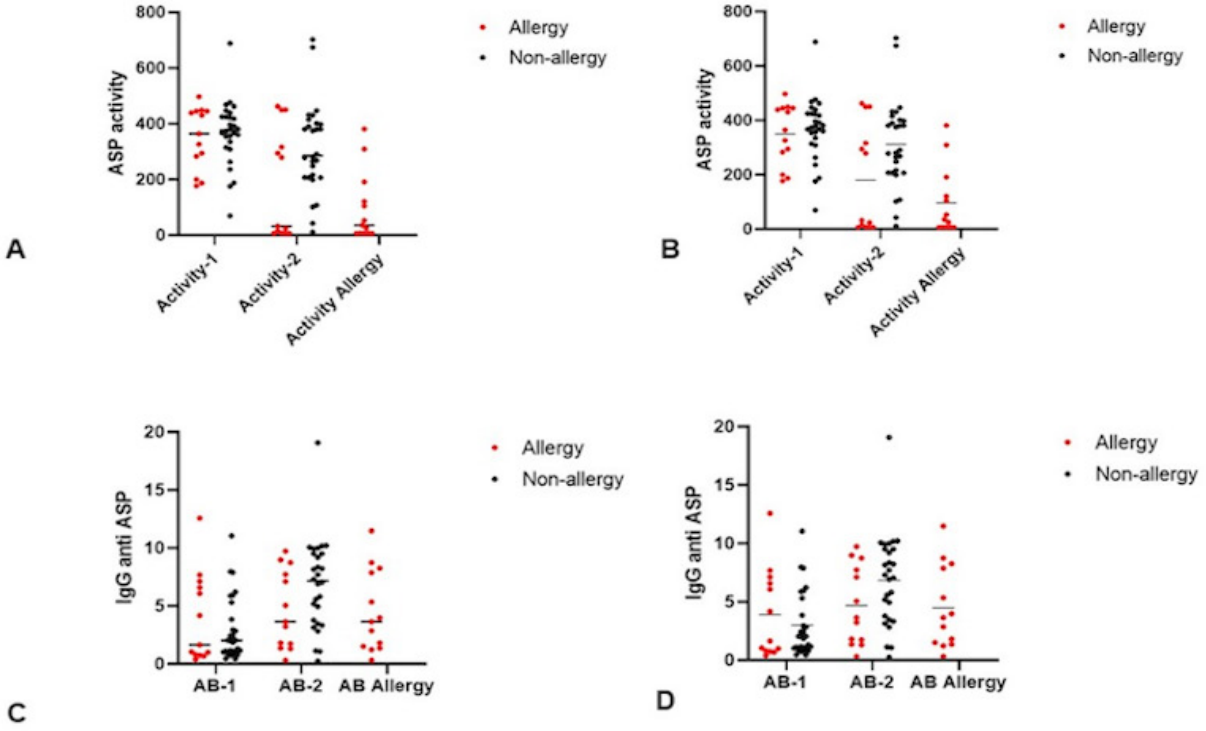

Figure 2. Scattered Diagram Antibody and Activity *E. coli* ASP. (A), ASP Activity (median) mIU/mL; (B), ASP Activity (mean) mIU/mL; (C), Antibody ASP (median) ng/mL; (D), Antibody ASP (mean) ng/mL *AB1: antibody initial induction, AB2: antibody end induction, AB: antibody allergy, ASP1: activity ASP initial induction, ASP2: activity end induction, ASP allergy: activity ASP allergy

Table 2. Association between the Initial and End of Induction Phase Anti-Asparaginase Antibody Levels in Allergy and Non-Allergy Groups

	Initial Induction	End Induction	P
Antibody levels Median (range) (ng/mL)			
Allergy (n=13)	1.65 (0.40 – 12.58)	3.64 (2.62 – 6.71)	>0.05[c]
Non-Allergy (n=28)	2.03 (0.44 – 11.03)	7.16 (0.24 – 19.08)	<0.001[d]
Activity levels Median (range) (mIU/mL)			
Allergy (n=13)	364.34 (176.82 – 497.19)	32.51 (5.4-462.4)	>0.05[d]
Non-Allergy (n=28)	376.21 (69.73 – 688.50)	285.64 (10.70 – 702.45)	>0.05[c]

[c], calculated by independent T-test; [d], calculated by Mann-Whitney

common onset of allergic manifestations was at the 5th dose, with a total of 6–8 doses of ASP being given. In the most recent ALL protocols worldwide, ASP is used in the induction and re-induction phases. Allergic manifestations generally occur in the post-induction or re-intensification phase, after a relatively long rest period (hiatus) (Woo et al., 1998; Woo et al., 2000; Szewczyk et al., 2004; Szewczyk BZ et al., 2007; Ebeid et al., 2008; Schrey et al., 2010; Liu et al., 2012; Chen, 2015; Ko et al., 2015; Walenciak et al., 2019). All allergic manifestations in this study occurred within 15-30 minutes of asparaginase infusion in the induction phase, including rapid allergic reactions, which have typical the type 1 hypersensitivity mechanism (Warrington et al., 2011; Franceschini et al., 2019).

Type 1 hypersensitivity or allergic reactions in this study occurred after less intensive exposure to ASP than in other studies, did not detect anti-ASP IgG and IgM antibodies at the end of induction, after 8 doses of ASP at a dose of 5,000 IU/m^2 (Szewczyk et al., 2004). The IgG levels at the three measurement times (initial-allergy-end induction) in this study are lower than the category threshold used in a Polish study (>31.67 µg/mL) (Walenciak et al., 2019). Interestingly, even though a rising trend was observed in both groups, there was no significant increase in IgG levels in the allergy subgroup. The role of IgG antibodies in allergic reactions has been described in numerous studies, while only a few studies were conducted in induction phase due to less frequent allergy manifestation (Woo et al., 1998; Woo et al., 2000; Szewczyk et al., 2004; Szewczyk BZ et al., 2007; Ebeid et al., 2008; Schrey et al., 2010; Liu et al., 2012; Chen, 2015; Ko et al., 2015; Walenciak et al., 2019).

Based on the adequate level of ASP *E. coli* activity at 24 hours of >100 mIU/ml, the non-allergy group had a significantly higher proportion of adequate activity levels

compared to the allergy group (p=0.001, Table 1), although the decrease in activity levels at the end of induction in the allergy group was not statistically significant (p=0.389, Table 2). The ASP *E. coli* activity during allergy was not associated with an increase in IgG anti-ASP positivity. These results are in contrast to previous studies in which allergy manifestations were associated with the detection of anti-ASP antibodies and decreased ASP activity(Fernandez et al., 2015; Nogami-Hara et al., 2016; Rodríguez et al., 2017). This was probably due to the smaller number of *E. coli* ASP administrations before the onset of allergy compared to these studies in literature, and possibility other immunological mechanisms of *E. coli* ASP allergy manifestations in induction phase of ALL treatment (Schrey et al., 2010; Walenciak et al., 2019).

E. coli asparaginase is a large molecular weight protein that has the potential to stimulate the human body to produce anti-drug-antibodies, which is associated with treatment failure, because the neutralizing antibodies inhibit the pharmacological function of ASP (Franceschini et al., 2019). The types of neutralizing antibodies induced by the administration of ASP might include IgG, IgM, and IgE (Szewczyk et al., 2004; Szewczyk BZ et al., 2007; Fernandez et al., 2015; Nogami-Hara et al., 2016; Rathod et al., 2019; Walenciak et al., 2019). In this study, all the patients with an allergy experienced this within one hour of ASP infusion. This reflects the possibility of rapid reactions caused by direct activation of mast cells, known as IgE-mediated hypersensitivity (by IgE antibodies) (Franceschini et al., 2019). Indeed, premedication with antihistamines and dexamethasone in this allergy group prevented subsequent allergic manifestations supporting the role of IgE in asparaginase allergy (Fernandez et al., 2015). IgE is known to cause type I hypersensitivity reactions to occur rapidly, generally within 1 hour after exposure, and can be prevented by administering non-anaphylactoid IgE antibodies, as demonstrated in animal studies by Hara et al (Nogami-Hara et al., 2016). The involvement of IgM antibodies in the induction phase causing neutralization of ASP is greater than by other immunoglobulin isotypes based on the Walenciak study (Walenciak et al., 2019). They observed a higher increase of IgM antibodies in the group with low ASP activity in the induction phase, whereas there was a weak and moderate negative correlation between IgM and IgG anti-ASP with ASP activity in the re-induction phase (Walenciak et al., 2019). Previously, Zalewska et al. observed the formation of IgM antibodies in a group of allergy patients, and the level of positive IgM antibodies was higher in the induction phase than in the re-induction phase, while IgG antibody levels were higher in the re-induction phase (Szewczyk et al., 2004; Szewczyk BZ et al., 2007). The same authors also reported that the group with antibodies had lower ASP activity. When administering PEG-ASP, 100% IgG and 67% IgM antibodies were detected (Kloos et al., 2020). The predominant type of antibody is related to the time of exposure because, in the process of antigen immunity, there is a transition of antibodies produced by B-cells (Abbas et al., 2018). This has not been proven in ASP studies at the molecular level, but referring to the adaptive immune response, IgM is an effector antibody produced from Th_1 when stimulated by antigen-presenting cells and has the potential to play a role in neutralizing antibodies (Abbas et al., 2018).

Further studies with larger patient numbers should be conducted to confirm this study result, including evaluation of Ig E and Ig M anti *E. coli* ASP antibodies, at the different time points during induction. Meanwhile, in clinical practice and especially in limited resources settings with lack of alternative ASP preparations, it seems safe in general to continue giving ASP infusion after allergy events (non-severe allergy) with proper prophylaxis medication and a switch to intramuscular administration, to prevent severe allergy. However, in patients in whom inadequate ASP activity is measured, further administration of the *E. coli* ASP seems useless.

In conclusions, adequate ASP *E. coli* activity was higher in non-allergy group than in allergy group. ASP *E. coli* activity levels were not associated with the level of IgG anti ASP *E. coli* antibodies, which indicates that there are other factors that may affect activity levels in the allergy group such as Ig E or IgM antibodies. Alternatively, we may not have detected the relevant IgG antibodies. The ASP *E. coli* activity level in the allergy group at the end of induction did not show a significant decrease from the induction phase.

Author Contribution Statement

NMS, AB, SS, LR, PI concepted, designed and developed research method and data analysis. All the authors wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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Ethical Approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards". The study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran with the issuance of the ethical clearance no. 469/UN6.KEP/EC/2019

Availability of Data

The data analyzed in this study are subject to the licenses/restrictions. Requests to access these datasets should be directed to the corresponding authors

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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