

## BAB VII

### KESIMPULAN DAN SARAN

#### 7.1 Kesimpulan

1. *Mixed infection Blastocystis* ST 1 dan ST 3 pada tikus menyebabkan terjadinya inflamasi ringan-sedang pada pemeriksaan histopatologi jaringan usus, yang ditandai dengan ditemukannya infiltrasi sel leukosit, edema sub mukosa, penurunan jumlah sel goblet dan kerusakan epitel. Perubahan ini meningkat secara bermakna pada kelompok tikus yang diberi perlakuan dosis  $10^5$  ( $p < 0,05$ )
2. *Mixed infection Blastocystis* ST 1 dan ST 3 pada tikus menyebabkan terjadinya peningkatan ekspresi protein NF- $\kappa$ B. Peningkatan ini terjadi secara bermakna pada kelompok tikus yang diberi perlakuan dosis  $10^5$  ( $p < 0,05$ )
3. *Mixed infection Blastocystis* ST 1 dan ST 3 pada tikus menyebabkan terjadinya peningkatan ekspresi protein caspase 3, yang menggambarkan terjadinya peningkatan apoptosis sel epitel usus. Peningkatan ini terjadi secara bermakna pada kelompok tikus yang diberi perlakuan dosis  $10^5$  ( $p < 0,05$ )
4. *Mixed infection Blastocystis* ST 1 dan ST 3 pada tikus menyebabkan terjadinya penurunan ekspresi protein dan ekspresi gen ZO-1, yang menggambarkan terjadinya kerusakan *tight junction* pada jaringan usus. Penurunan ini terjadi secara bermakna pada kelompok tikus yang diberi perlakuan dosis  $10^5$  ( $p < 0,05$ )

## 7.2 Saran

1. Perlu dilakukan penelitian selanjutnya untuk melihat pengaruh *Blastocystis sp* dengan dosis yang lebih tinggi dan infeksi yang lebih lama.
2. Perlu dilakukan penelitian selanjutnya untuk melihat ekspresi mRNA NF- $\kappa$ B dan caspase 3 untuk mengkonfirmasi hasil penelitian ini.
3. Perlu dikembangkan penelitian terkait penilaian jaringan usus manusia yang terinfeksi *Blastocystis sp* dengan pemeriksaan kolonoskopi dan biopsi sehingga bisa dijadikan marker atau deteksi dini perubahan kearah IBS atau kanker kolon.
4. Perlu dilakukan penelitian lanjutan untuk menemukan modalitas terapi yang lebih akurat, sesuai dengan patogenesis yang telah dibuktikan.
5. Kepada klinisi dapat disarankan supaya dilakukan pemeriksaan *Blastocystis* pada pasien IBS dan IBD



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## Lampiran 1. Surat persetujuan etik



KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,  
RISET DAN TEKNOLOGI  
UNIVERSITAS ANDALAS  
FAKULTAS KEDOKTERAN  
**KOMISI ETIK PENELITIAN**

Alamat : Kampus Universitas Andalas, Limau Manis Padang Kode Pos 25163  
Telepon : 0751-31746, Faksimile : 0751-32838, Dekan : 0751-39844  
Laman : <http://fk.unand.ac.id> e-mail : [dekanat@fk.unand.ac.id](mailto:dekanat@fk.unand.ac.id)

**KETERANGAN LOLOS KAJI ETIK**  
**DESCRIPTION OF ETHICAL APPROVAL**

No : 52/UN.16.2/KEP-FK/2023

Tim Komisi Etik Penelitian Fakultas Kedokteran Universitas Andalas, dalam upaya melindungi Hak Azasi dan Kesejahteraan Subjek Penelitian kedokteran/kesehatan, telah mengkaji dengan teliti protokol penelitian dengan judul :

*(The Research Ethics Committee Faculty of Medicine Universitas Andalas, in order to protect human rights and welfare of medical/health research subject, has carefully reviewed the research protocol entitled) :*

**Kajian Gen Zonula Occludin 1 (ZO1), Protein Nuclear Factor Kappa Beta (NFkB) dan Apoptosis Sel Epitel Usus Akibat Infeksi Blastocystis Subtipe 1**

Nama Peneliti Utama : dr. Eka Nofita, M.Biomed  
*Principal Researcher*

Nama Institusi : Program Studi Ilmu Biomedis Program Doktor  
FK Universitas Andalas  
*Institution*

**Protokol Penelitian tersebut dapat disetujui pelaksanaannya**  
*and approved the research protocol.*

Padang, 23 Februari 2023

Dekan Fakultas Kedokteran Universitas Andalas  
*Dean of Faculty of Medicine Universitas Andalas*

Dr. dr. Afriwardi, SH. Sp.KO, MA  
NIP 196704211997021001

Ketua  
*Chairman*

Dr. dr. Yuliarni Syafrita, Sp.S (K)  
NIP 196407081991032001

**Keterangan/notes:**

Keterangan lolos kaji etik ini berlaku satu tahun dari tanggal persetujuan.

*This ethical approval is effective for one year from the due date.*

Jika ada kejadian serius yang tidak diinginkan (KTD) harus segera dilaporkan ke Komisi Etik Penelitian.

*If there are Serious Adverse Events (SAE) should be immediately reported to the Research Ethics Committee.*

Lampiran 2. Kriteria *Bethel Manja*

Tabel 2.1 Skor ditemukan udem pada submukosa

Skor	Interpretasi
0	Tidak ditemukan perubahan patologi
1	Udem ringan(pelebaran udem <50% dari luas dinding usus)
2	Udem sedang (ditemukan udem sekitar 0,21-0,45mm)
3	Udem dengan kedalaman (ditemukan udem pada mukosa selebar >0,46 mm)

Tabel 2.2 Skor ditemukan infiltrasi PMN ke arah lamina propria

Skor	Interpretasi
0	<5 PMN
1	5-20 PMN
2	21-60 PMN
3	61-100 PMN
4	>100 PMN

Tabel 2.3 Skor ditemukan jumlah sel goblet

Skor	Interpretasi
0	>28 sel goblet
1	11-28 sel goblet
2	1-10 sel goblet
3	<1 sel goblet

Tabel 2.4 Skor menentukan kerusakan epitel

Skor	Interpretasi
0	tidak ada perubahan histologi epitel
1	deskuamasi epitel
2	erosi epitel
3	ulserasi epitel

Didapatkan kesimpulan akhir, bahwa derajat inflamasi dinyatakan dalam bentuk nilai rata-rata antara 0 dan 13.

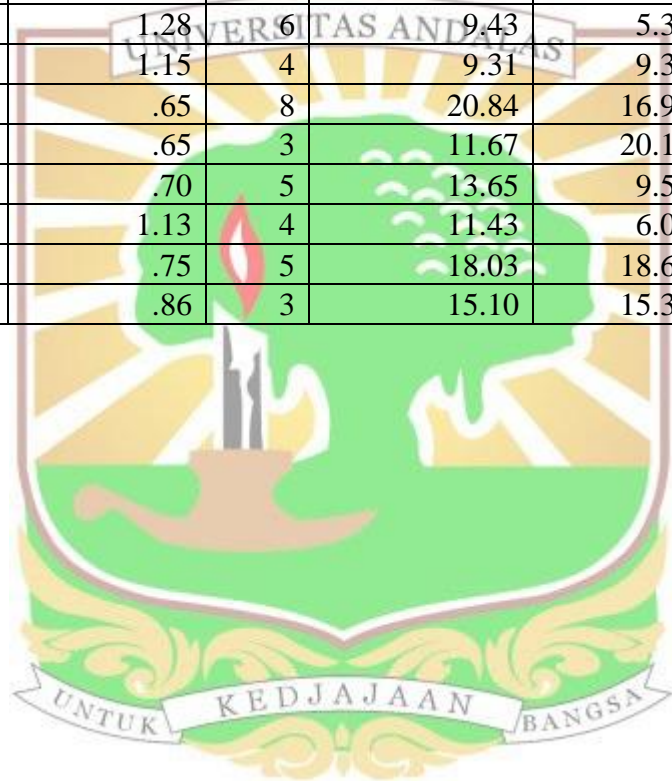
Tabel 2.5 Interpretasi derajat inflamasi pada usus

Skor	Derajat inflamasi
0	Tidak ditemukan tanda-tanda inflamasi
1-2	Ditemukan tanda inflamasi yang relative minimal
3-4	Derajat ringan
5-8	Derajat sedang
9-13	Ditemukan tanda-tanda inflamasi yang berat dan dalam



Lampiran 3. Master Tabel

kode	grup	RTPCR.ZO1	BM	IHK.Apoptosis	IHK.NFkB	IHK.ZO1
15	1	1.00	1	9.00	8.63	43.63
16	1	1.36	3	8.72	5.62	38.45
23	1	1.03	2	9.57	6.57	41.63
31	1	.77	3	7.39	8.38	55.78
50	1	2.10	2	7.62	8.32	33.63
52	1	1.54	2	9.74	6.94	32.52
4	2	1.42	4	9.70	9.37	39.81
5	2	1.56	4	12.73	12.44	42.49
7	2	2.53	3	12.37	7.49	27.83
8	2	1.32	4	8.47	5.43	31.61
9	2	1.28	6	9.43	5.37	28.85
10	2	1.15	4	9.31	9.34	32.51
1	3	.65	8	20.84	16.95	22.18
2	3	.65	3	11.67	20.16	21.13
3	3	.70	5	13.65	9.52	18.37
12	3	1.13	4	11.43	6.09	32.43
13	3	.75	5	18.03	18.66	22.21
14	3	.86	3	15.10	15.34	32.14





## Lampiran 4. Hasil pemeriksaan nanodrop

Data Nanodrop RNA									Pengenceran RNA			Sintesis cDNA			
NO	Sample ID	Nucleic Acid Conc.	Unit	260/280	Nucleic Acid Conc.	Unit	260/280	Mean RNA Conc.	Vol. RNA utk Pengenceran RNA 100 ng/ul	Vol. NFW utk pengenceran RNA	Konsentrasi Akhir RNA setelah pengenceran (ng/ul)	Vol RNA (1 µg RNA)	5x Trans Amp Buffer	Reverse Transcriptase	Nuclease-free water
1	1	527.5	ng/µl	1.93		ng/µl	1.93	527.5	3.8	16.2	100.0	10.0	4	1	5
2	2	1642.3	ng/µl	1.95	1642.3	ng/µl	1.95	1642.3	1.2	18.8	100.0	10.0	4	1	5
3	3	1924.5	ng/µl	1.91	1924.5	ng/µl	1.91	1924.5	1.0	19.0	100.0	10.0	4	1	5
4	4	1763.8	ng/µl	1.92	1763.8	ng/µl	1.92	1763.8	1.1	18.9	100.0	10.0	4	1	5
5	5	1032.9	ng/µl	1.94	1032.9	ng/µl	1.94	1032.9	1.9	18.1	100.0	10.0	4	1	5
6	7	852.5	ng/µl	1.99	852.5	ng/µl	1.99	852.5	2.3	17.7	100.0	10.0	4	1	5
7	8	1792.5	ng/µl	1.92	1792.5	ng/µl	1.92	1792.5	1.1	18.9	100.0	10.0	4	1	5
8	9	1014.5	ng/µl	1.97	1014.5	ng/µl	1.97	1014.5	2.0	18.0	100.0	10.0	4	1	5
9	10	2077.2	ng/µl	1.88	2077.2	ng/µl	1.88	2077.2	1.0	19.0	100.0	10.0	4	1	5
10	12	2062.9	ng/µl	1.88	2062.9	ng/µl	1.88	2062.9	1.0	19.0	100.0	10.0	4	1	5
11	13	730	ng/µl	1.94	730	ng/µl	1.94	730	2.7	17.3	100.0	10.0	4	1	5
12	14	1764.9	ng/µl	1.92	1764.9	ng/µl	1.92	1764.9	1.1	18.9	100.0	10.0	4	1	5
13	15	1769.5	ng/µl	1.92	1769.5	ng/µl	1.92	1769.5	1.1	18.9	100.0	10.0	4	1	5
14	16	1254.7	ng/µl	1.86	1254.7	ng/µl	1.86	1254.7	1.6	18.4	100.0	10.0	4	1	5
15	23	1169.5	ng/µl	1.91	1169.5	ng/µl	1.91	1169.5	1.7	18.3	100.0	10.0	4	1	5
16	31	2289.2	ng/µl	1.88	2289.2	ng/µl	1.88	2289.2	0.9	19.1	100.0	10.0	4	1	5
17	50	2906.6	ng/µl	1.95	2906.6	ng/µl	1.95	2906.6	0.7	19.3	100.0	10.0	4	1	5
18	52	1401.3	ng/µl	1.88	1401.3	ng/µl	1.88	1401.3	1.4	18.6	100.0	10.0	4	1	5

## Lampiran 5. Hasil SPSS

**1. SPSS gambaran histopatologi berdasarkan skor skor barthel manja**

## A. Uji normalitas skor barthel manja

Tests of Normality							
	grup penelitian	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	kontrol	.254	6	.200*	.866	6	.212
BM	P1	.401	6	.003	.770	6	.031
	P2	.262	6	.200*	.862	6	.195

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

## B. Uji Kruskal Wallis Skor BM

Test Statistics <sup>a,b</sup>	
	BM
Chi-Square	10.045
Df	2
Asymp. Sig.	.007

a. Kruskal Wallis Test

b. Grouping Variable:  
grup penelitian

## C. Uji Tukey

## Multiple Comparisons

Dependent Variable: BM

Tukey HSD

(I) grup penelitian	(J) grup penelitian	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	P1	-2.000*	.745	.042	-3.94	-.06
	P2	-2.500*	.745	.011	-4.44	-.56
P1	kontrol	2.000*	.745	.042	.06	3.94
	P2	-.500	.745	.784	-2.44	1.44
P2	kontrol	2.500*	.745	.011	.56	4.44
	P1	.500	.745	.784	-1.44	2.44

\*. The mean difference is significant at the 0.05 level.

**2. SPSS IHK NFKB**

## A. Uji normalitas

## Tests of Normality

	grup penelitian	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	Control	.273	6	.184	.891	6	.322
IHK.NFkB	P1	.183	6	.200*	.918	6	.493
	P2	.231	6	.200*	.912	6	.447

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

## B. Uji One way Anova

## Descriptives

IHK.NFkB

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
					control	6		
P1	6	8.2400	2.71376	1.10789	5.3921	11.0879	5.37	12.44
P2	6	14.4533	5.50572	2.24770	8.6754	20.2312	6.09	20.16
Total	18	10.0344	4.68779	1.10492	7.7033	12.3656	5.37	20.16

## Test of Homogeneity of Variances

IHK.NFkB

Levene Statistic	df1	df2	Sig.
6.153	2	15	.011

## ANOVA

IHK.NFkB

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	177.806	2	88.903	6.812	.008
Within Groups	195.775	15	13.052		
Total	373.581	17			

Test Statistics<sup>a,b</sup>

	IHK.NFkB
Chi-Square	6.035
df	2
Asymp. Sig.	.049

a. Kruskal Wallis Test

b. Grouping Variable: grup penelitian

## C. Uji tukey

### Multiple Comparisons

Dependent Variable: IHK.NFkB

Tukey HSD

(I) grup penelitian	(J) grup penelitian	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	P1	-.83000	2.08580	.917	-6.2478	4.5878
	P2	-7.04333*	2.08580	.011	-12.4611	-1.6255
P1	Control	.83000	2.08580	.917	-4.5878	6.2478
	P2	-6.21333*	2.08580	.024	-11.6311	-.7955
P2	Control	7.04333*	2.08580	.011	1.6255	12.4611
	P1	6.21333*	2.08580	.024	.7955	11.6311

\*. The mean difference is significant at the 0.05 level.

### 3. SPSS IHK caspase 3

#### A. Uji normalitas

##### Tests of Normality

	grup penelitian	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	Df	Sig.
IHK.Apoptosis	Control	.192	6	.200*	.903	6	.390
	P1	.307	6	.080	.839	6	.129
	P2	.169	6	.200*	.921	6	.512

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

#### B. Uji Homogenitas

##### Test of Homogeneity of Variances

IHK.Apoptosis

Levene Statistic	df1	df2	Sig.
4.480	2	15	.030

##### Descriptives

IHK.Apoptosis

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
kontrol	6	8.6733	.98052	.40030	7.6443	9.7023	7.39	9.74
P1	6	10.3350	1.76796	.72177	8.4796	12.1904	8.47	12.73
P2	6	15.1200	3.71124	1.51511	11.2253	19.0147	11.43	20.84
Total	18	11.3761	3.62780	.85508	9.5720	13.1802	7.39	20.84

##### ANOVA

IHK.Apoptosis

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	134.434	2	67.217	11.290	.001
Within Groups	89.302	15	5.953		

Total	223.736	17			
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**Test Statistics<sup>a,b</sup>**

	IHK.Apoptosis
Chi-Square	10.398
df	2
Asymp. Sig.	.006

a. Kruskal Wallis Test

b. Grouping Variable: grup penelitian

## C. Uji Tukey

**Multiple Comparisons**

Dependent Variable: IHK.Apoptosis

Tukey HSD

(I) grup penelitian	(J) grup penelitian	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	P1	-1.66167	1.40872	.483	-5.3208	1.9974
	P2	-6.44667*	1.40872	.001	-10.1058	-2.7876
P1	Control	1.66167	1.40872	.483	-1.9974	5.3208
	P2	-4.78500*	1.40872	.010	-8.4441	-1.1259
P2	Control	6.44667*	1.40872	.001	2.7876	10.1058
	P1	4.78500*	1.40872	.010	1.1259	8.4441

\*. The mean difference is significant at the 0.05 level.

**4. SPSS IHK Z01**

## A. Uji normalitas

**Tests of Normality**

	grup penelitian	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
IHK.Z01	Control	.209	6	.200*	.908	6	.425

P1	.255	6	.200*	.888	6	.309
P2	.330	6	.040	.816	6	.081

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

### Descriptives

IHK.ZO1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
					kontrol	6		
P1	6	33.8500	5.97034	2.43738	27.5845	40.1155	27.83	42.49
P2	6	24.7433	6.00765	2.45261	18.4387	31.0480	18.37	32.43
Total	18	33.1778	9.41906	2.22009	28.4938	37.8618	18.37	55.78

### Test of Homogeneity of Variances

IHK.ZO1

Levene Statistic	df1	df2	Sig.
.191	2	15	.828

### ANOVA

IHK.ZO1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	791.063	2	395.531	8.273	.004
Within Groups	717.155	15	47.810		
Total	1508.218	17			



### Multiple Comparisons

Dependent Variable: IHK.ZO1

LSD

(I) grup penelitian	(J) grup penelitian	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	P1	7.09000	3.99209	.096	-1.4189	15.5989
	P2	16.19667*	3.99209	.001	7.6877	24.7056
P1	Control	-7.09000	3.99209	.096	-15.5989	1.4189

	P2	9.10667*	3.99209	.038	.5977	17.6156
P2	Control	-16.19667*	3.99209	.001	-24.7056	-7.6877
	P1	-9.10667*	3.99209	.038	-17.6156	-.5977

\*. The mean difference is significant at the 0.05 level.

## 5. SPSS RT PCR ZO1

### Tests of Normality

	grup penelitian	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
RTPCR.ZO1	kontrol	.214	6	.200*	.937	6	.636
	P1	.320	6	.054	.752	6	.021
	P2	.253	6	.200*	.817	6	.083

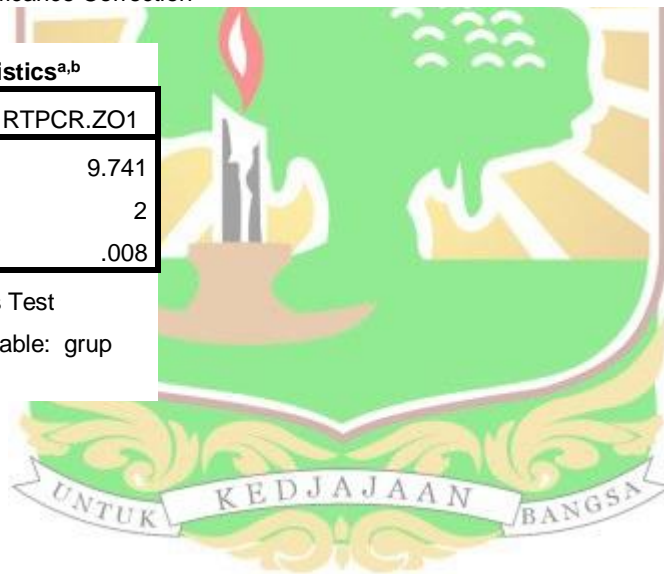
\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Test Statistics <sup>a,b</sup>	
	RTPCR.ZO1
Chi-Square	9.741
df	2
Asymp. Sig.	.008

a. Kruskal Wallis Test

b. Grouping Variable: grup penelitian



### Descriptives

RTPCR.ZO1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
kontrol	6	1.3000	.47854	.19536	.7978	1.8022	.77	2.10
P1	6	1.5433	.50258	.20518	1.0159	2.0708	1.15	2.53
P2	6	.7900	.18407	.07514	.5968	.9832	.65	1.13
Total	18	1.2111	.50591	.11924	.9595	1.4627	.65	2.53

### Multiple Comparisons

Dependent Variable: RTPCR.ZO1

LSD

(I) grup penelitian	(J) grup penelitian	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol	P1	-.24333	.23932	.325	-.7534	.2668
	P2	.51000	.23932	.050	-.0001	1.0201
P1	Control	.24333	.23932	.325	-.2668	.7534
	P2	.75333*	.23932	.007	.2432	1.2634
P2	Control	-.51000	.23932	.050	-1.0201	.0001
	P1	-.75333*	.23932	.007	-1.2634	-.2432

\*. The mean difference is significant at the 0.05 level.





## Lampiran 6. Dokumentasi

 <p>Pemeliharaan tikus</p>	 <p>Inokulasi <i>Blastocystis sp.</i> pada tikus secara oral</p>
 <p>Penimbangan berat badan dan pengamatan terhadap tikus</p>	 <p>Pengambilan organ sekum dan kolon tikus</p>
 <p>Organ sekum dan kolon tikus</p>	 <p>Pewarnaan IHK</p>