Parameters of Systemic Inflammatory Response in Patients with Passing Infringements of Brain Blood Circulation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NVH and MNS designed the study, interpreted the data and wrote the first draft of the manuscript. Author NVH carried out the patient recruitment and data collection. Authors EVV and NMH performed all the laboratory research. Authors VBS and EVV managed the study. Authors MNS and SVS conducted a statistical analysis of the data. All authors read and approved the final manuscript.

Short Research Article

ABSTRACT

Aims: The paper aims at revealing the difference in the change of Systemic Inflammation (SI) parameters in blood plasma of patients with Passing Infringements of Brain Blood Circulation (PIBBC) and stroke during 10-day acute period.
Study Design: Prospective cohort study.
Place and Duration of Study: Department of Neurology and Neurosurgery of the Gomel State Medical University, Stroke Unit of the Gomel Regional Veterans Hospital, between May 2013 and March 2015.
Methodology: This study included 108 patients (35 men, 73 women; age range is 46-90 years) and 20 volunteers over 45 years (11 men, 9 women, mean age is 54.3±1.6 years). We compared

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the parameters of systemic inflammation (blood plasma concentrations of interleukins (IL-6, IL-8, IL-10), tumor necrosis factor α (TNFα), C-reactive protein (CRP) and stable metabolites of nitric oxide (nitrite- and nitrate-ions, NO_x) in patients with PIBBC including transient ischemic attack (TIA) and cerebral hypertensive crisis (CHC), patients with lacunar stroke (LS) on the first and tenth day of patient staying in the hospital and in volunteers of a control group.

Results: On the first day, the IL-6 and CRP levels for PIBBC and LS groups was found to be significantly higher than the corresponding parameters for a control group. An increase in IL-6 level during the 10-day therapeutic period was observed only in blood plasma of the LS group persons. The TNF-α level was about zero level for all the studied groups except for the LS group on the tenth day. On the first day, for the patients with LS the IL-8 level was significantly decreased comparing to that for the control group and PIBBC groups and the NO_x level was significantly lower than that for CHC group. Both parameters increased in values during the 10-day therapeutic period. No difference between the parameters (NO_x and IL-8) for PIBBC and control groups was detected on the first day. Coefficient of reactivity characterizing the systemic inflammation level in patients of all the studied groups was nonzero level and it increased in the LS patients during the therapeutic period.

Conclusion: The obtained data showed an important role a number of inflammation markers (NOx, IL-6, IL-8, TNF-α, CRP) in the pathogenesis of different stroke episodes. Absence of specific neuroimaging changes in TIA and CHC group and nonzero coefficient of reactivity (k_R) suggests that systemic inflammation is not a result of the focal brain ischemia but its intrinsic cause. The differences in the concentration of IL-8, and NO_x in the blood plasma of patients with CHC and LS on their admission to the hospital will help to identify sanogenetic reserves in the case of PIBBC.

1. INTRODUCTION

Stroke is the second leading cause of death for people in Europe and first cause of the disability in age after 60 years [1,2]. Passing infringements of brain blood circulation (PIBBC) including transient ischemic attack (TIA) and cerebral hypertensive crisis (CHC) often precede the stroke [1,3]. TIA patients constitute a high risk group for subsequent stroke with an early risk of 4-8% and 30% 1 month and 5 years after cerebrovascular event respectively [1,3]. Diagnosis of PIBBC is mainly based on clinical criteria only [1,4]. Biochemical parameters ("biomarkers") that characterize TIA and CHC pathological mechanisms are able to help clarify the diagnosis, improve the understanding of therapeutic strategies and estimation of the risk for stroke after PIBBC.

Biomarkers of the aseptic inflammation are found in the blood, other body fluids or tissues long before clinical symptoms of blood circulation disruption. Therefore, panels of biomarkers are used for the analysis of complex inflammatory processes. In study by Gusev et al. [5], the degree of systemic inflammation (SI) was characterized by reactivity coefficient (CR) calculated using five parameters (concentrations of interleukins 6 (IL-6), 8 (IL-8) and 10 (IL-10),

tumor necrosis factor α (TNF-α) and C-reactive protein (CRP). In our work, this panel of biomarkers was used for the SI study in patients with different PIBBC forms and stroke.

The increased values of SI parameters in the cerebrospinal fluid of patients with stroke and TIA were previously reported [6]. It was noted that only the levels of IL-6 and N-terminal pro-brain natriuretic peptide in patients with heart attack or TIA at admission had a prognostic significance in adverse outcomes [6,7,8]. A positive correlation was found between the level of the blood plasma pro-inflammatory cytokines, fibrinogen and glucose levels, body temperatures and poor outcome [9,10]. That fact was also confirmed in various stroke subtypes according to TOAST classification [11]. IL-6 level is considered as a parameter of early inflammatory response during heart attack [12]. The increase in IL-6 level in blood of TIA patients recently demonstrated in literature [12,13,14] was correlated with the results of MRI diffusion and had a prognostic value. IL-6 plays an important role of in the genesis of arterial thrombosis. The question of whether IL-6 is involved in the pathogenesis of myocardial infarction or determines the cellular pathways during the early inflammatory process is broadly discussed in literature [9,15,16]. IL-10 is supposed to be an early predictor of the

outcome of heart attack [6,16]. One of important roles of IL-10 is to inhibit excessive production of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12 and TNF-α [17]. According to an alternative hypothesis, the increase of IL-6, IL-10 and CRP levels can be an early predictor of infectious complications after heart attack [16]. However, Kumar D. et al. did not find a link between IL-6 and IL-10 levels in the acute period of the stroke in immunosuppressive patients [18]. Additionally to antiinflammatory effect, IL-10 has an antiapoptotic effect by way of suppressing the antigen presenting cell activity [19]. IL-10 stimulates the production of monocyte soluble forms of TNF-α receptor antagonist and IL-1 [19]. IL-10 level is correlated to the prognosis of functional recovery after acute cerebral ischemia [20]. IL-8 is known to be a proinflammatory cytokine in acute and chronic inflammatory processes. Its main effects are the induction of chemotaxis of neutrophils, eosinophils, basophils and monocytes as well as stimulation of angiogenesis [20]. IL-8 production increases under the action of TNF-α and IL-1, and under the oxidative stress condition [20]. TNF-α is a proinflammatory cytokine that level increases during the first 1-3 hours after the stroke [21,22]. The second peak of TNF-α evaluation is observed after next 24-36 hours [23,24]. Performing pleiotropic functions and having a proinflammatory potential, TNF-α mediates the tissue protection to hypoxic injury [25,26]. TNF-α plays also important role of in angiogenesis [27] and increase of the necrosis volume after heart attack [21]. The high-sensitivity C-reactive protein [8,28-35] (CRP) is an acute phase inflammatory marker. The synthesis of the protein is regulated by IL-1 and IL-6. CRP is responsible for nonspecific autoimmune response [36]. Baseline CRP level less than 0.5 mg/L is used as a prognostic indicator in cardiovascular pathology and re-stroke, particularly in conjunction with high level of troponin, fibrinogen and cholesterol [36]. A diagnostic [31] and prognostic [9,37,38,39] significance of CRP level determination for the estimation of the risk of myocardial infarction and stroke in TIA patients was demonstrated in literature [32,33,34,37,40]. However, other researchers had shown the low effectiveness of the determination of only CRP level for prognosis of stroke [30,40,41,42,43,44,45]. In 2005, the National Heart Association (USA) was not officially recommended the routine screening CRP test for diagnosis and prognosis of stroke and TIA [44]. Further researches are needed to clarify CRP significance [45,46,47]. Nitric oxide

(NO) plays an important role in a wide variety of physiological processes including vascular relaxation and inflammation. Excessive production of reactive oxygen species (ROS) including NO are associated with ischemic stroke and other neurodegenerative diseases [48]. A relationship between CRP blood level and NO production [49] in stroke and in PIBBC [46] was shown. To assess NO production in tissues and liquids of an organism is broadly used the measuring of the concentration of the NO stable end products, nitrite and nitrate (NOx).

The paper aims at revealing the difference in the change of SI parameters in blood plasma of patients with passing infringements of brain blood circulation (PIBBC) and stroke during 10-day acute period.

2. MATERIALS AND METHODS

Study design was the prospective cohort study. The 1st and 10th days for the analysis were chosen because the patients involved in our study had to stay in the hospital during 10 days according to the Clinical Protocols of diagnosis and treatment of neurological diseases in the Republic of Belarus. Clinical Protocol of the Republic of Belarus for the Management of Patients with Stroke and Transient Ischemic Attacks includes: Monitoring and correction of the blood pressure (Captoprili, Enalaprili), antiplatelet drugs (Acetylsalicylic acid, Dipyridamole), correction hypercholesterolemia, dyslipidemia (atorvastatin), neuroprotection (Emoxipine, Piracetam, Mildronat, Glycine).

2.1 Patients

108 stroke and PIBBC patients admitted in the Stroke Unit of the Gomel Regional Veterans Hospital were examined. The study involved 47 patients with TIA (damage is not visible on tomography) (mean age was 66,7±2,0 years, 28 women and 19 men.); 44 patients with CHC (mean age was 60,6±2,7 years, 35 women and 9 men), 17 patients with lacunar stroke (LS) (size of stroke < 15 mm; mean age was 68,2±2,8 years, 10 women and 7 men) and 20 volunteers over 45 years old (mean age was 54,3±1,6 years, 9 women and 11 men). All the patients and volunteers belonged to Caucasian race. Neurological deficit in TIA patients at admission was presented as hemiparesis (46.8%), aphasia (31.9%), instability in the Romberg position (76.6%), asymmetry of tendon reflexes and Babinski symptom (59.6%), hypoesthesia (34%).

Table 1. Major co morbidities for PIBBC and stroke patients (in %)

Co morbidity	Groups		
	TIA n=47	CHC n=43	LS n=14
TIA or CHC positive history	27.7	4.7	28.6
Hypertension I-II	51.1	46.5	42.9
Hypertension III	38.3	32.6	42.9
Ischemic heart disease	61.7	32,6	92.9
Heart attack	8.5	–	28.6
Diabetes mellitus	14.9	5.7	21.4
Atrial fibrillation	31.9	–	14.3
Nodular goiter	4.3	7.0	7.1
Peptic duodenal or gastric ulcer, chronic gastritis	2.1	26.2	7.1
Chronic cholecystitis,	2.2	16.3	7.1
Chronic hepatitis	2.2	14.0	-
Chronic pyelonephritis	2.2	9.3	14.3
Vascular pathology	8.5	9.3	7.1
Back pain	12.7	16.3	14.3

TIA subjects had the ABCD^2 score of 4.9; 4-6 (Mdn; 0.25-0.75). In patients with CHC we observed hemiparesis (14%), hypoesthesia (37.2%), instability in the Romberg position (74.1%), asymmetry of tendon reflexes (25.6%). The neurologic deficit in LS patients was objectified using the American National Institute of Health stroke scale (NIHSS). For LS patients the NIHSS score was 5; 3-7 on the 1st day and 2; 1-3 on the 10th day. The list of major comorbidities for PIBBC and stroke patients is presented in Table 1. Among the comorbidities in all groups prevailed hypertension, coronary heart disease and diabetes.

For the peoples of the control group somatic diseases were not identified. Exclusion criteria included severe neurological deficit (score>10 according to NIHSS), hemorrhagic stroke or subarachnoid bleeding, persons with the acute phase of chronic diseases. Catamnesis after the discharge of 22 patients from the hospital was formed on the base of telephone surveys and personal conversations. In period of 3 months after the patient discharge two persons had developed re-TIA (9%) and five persons had myocardial infarction (21.7%).

2.2 Biochemical Analysis

Venous blood samples were drawn at 8:00 AM on the 1st and 10th day of patient hospital staying. The same time conditions were fulfilled for the persons of the control group. Plasma levels of IL-6, IL-8, IL-10, TNF-α and CRPs were measured using a solid-phase chemiluminescent immunometric assay (Immunoassay Analyzer AMF M/ 340, a panel of reagents from "Vector-Best, Russia"). The upper limits of the normal levels are 5 pg/dL (IL-6), 10 pg/dl (IL-8), 5 pg/dl (IL-10), 8 pg/ml (TNF-α), 8 mg/l (CRP). Coefficient of reactivity (k_R) was calculated according to the tables from the Gusev's work [5]. Plasma concentration of NO_x (nitrate and nitrite ions) was measured using Griess reagent.

2.3 Statistical Analysis

Data are presented as median (Mdn), lower and upper quartiles (interquartile range (IQR): 0.25-0.75). Statistical analysis was performed using software "STATISTICA 7.0". U-Mann-Whitney and Wilcoxon analysis of variance was used to establish the difference between groups. To determine the prognostic value of the SI parameters the method of non-linear logistic regression was used.

3. RESULTS

The ranges of the SI parameters in healthy persons of old age were: 0 pg / mL (0.00-0.49 pg / ml) for IL-6; 6.50 pg / ml (5.29-7.72 pg / ml) for IL-8; 0 pg / ml for IL-10; 0 pg / ml for TNF-α; 4.8 mg / l (2.3-7.7 mg / l) for CRP [50]. The ranges of NO_x level for healthy persons of old age were estimated in our early work study using a sample with size of 206 (mean age was 57±2 years) [51]. It was 17.1 µM (11.8-26.1 µM).

The plasma levels of the studied parameters (IL-6, IL-8, CRP, NO_x) and their change during 10-day therapeutic course in patients with PIBBC

and LS are shown in Fig. 1. The concentrations two other cytokines (IL-10 and TNF-α) for all patient groups were below the test-sensitivity (Table 2). The only exception was the level of TNF-α for LS patients on the 10th day of patient staying in the hospital. The Fig. 1 presents the parameters obtained on admission (axis x) versus the corresponding parameters obtained on the discharge of the patient from the hospital (axis y). Chosen type of data presentation is convenient to the easy fixing of the increase (rose area) and decreases (white area) in the parameters during the therapeutic course as well the variation of the parameters among the studied groups.

As shown in the Fig. 1A, the IL-6 level for PIBBC and LS groups was significantly higher than that for control group ($p < 0.01$ for all the ischemic groups). However, there is no significant difference between the IL-6 plasma concentration for CHC and TIA groups. U-Mann-Whitney analysis showed that the IL-6 and CRP levels in LS patients were significantly increased during the 10-day therapeutic period (p=0.004, p=0.008) (Figs. 1A and 1B). The IL-6 level in LS patients was higher than in TIA patients (p=0.048) on admission (Fig. 1A).

Fig. 1B shows that the CRP level for TIA group was significantly higher than that for control group both on admission and on discharge (p=0.011, p=0.006) of the patients from the hospital and its interquartile range was similar to that for the LS group.

A significant decrease in the concentration of IL-8 (Fig. 1C) was detected in the plasma of patients with LS versus the parameter for TIA and CHC patients (p=0.002, p=0.006) on admission. After the therapeutic course, the IL-8 level in the plasma of LS patients remained lower than that in CHC patients.

No significant differences were found in IL-8 and NO_x levels between both PIBBC groups and control group (Figs. 1C and 1D). NO_x plasma concentrations increased during the therapeutic course in patients with LS and TIA. The significant difference was established between the NO_x levels for CHC and LS groups on admission only (p=0.049).

Parameter k_R for LS and PIBBC patients was above zero on admission (Table 2). For LS patient on the 10th day after their admission we found an increase of both statistical parameters for k_R: the median (Mdn) and interquartile range (0.25-0.75). The increase in the interquartile range was fixed also for parameter k_R of TIA patients on their discharge from the hospital.

Using the method of non-linear logistic regression we revealed that the IL-6 level on the 10th day was a significant predictor of re-TIA (OR = 0,86; $χ2 = 6,5$, p = 0.011). The IL-8 level on the 1st day (OR = 0,44; $χ2 = 4,5$, p = 0.033) and on the 10th day (OR = 0,2; $χ2 = 2,7$, p = 0.09) could be also considered as a predictor of re-TIA in 3-month follow-up. Among other laboratory parameters the blood plasma fibrinogen (OR = 0,28; $χ2 = 4,6$, p = 0.032) and blood plasma glucose levels on admission (OR = 0,51; $χ2 = 5,3$, p = 0.021) were of interest as prognostic factors.

4. DISCUSSION

Ischemic stroke is recently revealed to link to the phenomena of the endothelial dysfunction and endothelial activation [49,52,53]. Endothelial

Table 2. Plasma concentrations of IL-10,TNF-α and the coefficient of reactivity for the studied groups depending on the day of analysis

	Groups			
	CHC Mdn 0.25-0.75	**TIA** Mdn 0.25-0.75	**LS** Mdn 0.25-0.75	**control** Mdn 0.25-0.75
IL-10 1st day	0	0	0	0
IL-10 10th day	0	0	0	
TNF-α 1st day	0	0	0	0
TNF-α 10th day	0	0	0.0; 0.0-2.0	
k_R 1st day	1.0; 0.0-2.0*	1.0; 0.0-2.0*	1.0; 0.0-2.0*	0
k_R 10th day	1.0; 0.0-2.0*	1.0; 0.0-2.5*	2.0; 0.0-4.0*	

*Note * – p<0.05 comparing to control group;*

A

IL-6

B

CRP

C

IL-8

D

NO$_x$
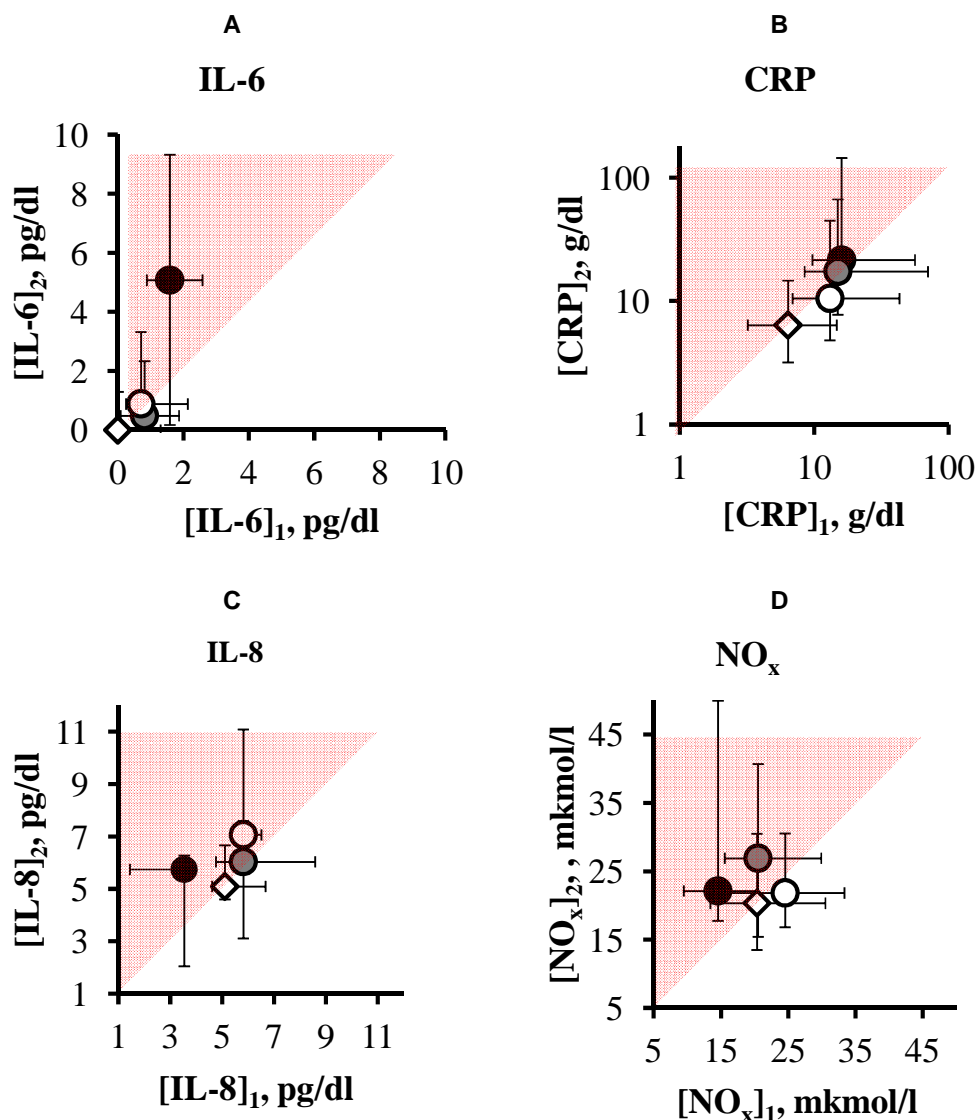
Fig. 1. The concentrations of IL-6 (A), CRP (B), IL-8 (C) and NO$_x$ (D) in the blood plasma in patients with LS (black circles), TIA (gray circles), CHC (white circles) and control subjects group (white diamonds) on admission in (axis x) and on discharge from (axis y) the stroke unit. The data for each axis are represented as Mdn and 0.25-0.75 IQR. The bisectrix divides the figure area into two parts: Area (in rose) of an increase and area (in white) of a decrease of the studied parameter values after 10-day therapeutic course

dysfunction is determined as the state of the endothelium with the decreased synthesis, release and activity of endothelium-derived NO [53]. Endothelial activation is a proinflammatory and procoagulant state of the endothelial cells characterized by an increase in their interaction with leukocytes and caused by proinflammatory cytokines, hypercholesterolemia, turbulent blood flows and others [54]. These two phenomena are tightly bound through the mechanisms with participation of NO, cytokines and other factors

including inflammatory mediators. Endothelial cell activation can lead to endothelial dysfunction by inhibiting endothelial NO-synthase and decreasing NO bioavailability through increasing in reactive oxygen species' production [54]. Our data have showed the difference in the endothelial cell behavior (the endothelial dysfunction and activation) related to LS and PIBBc types. The fact that LS was associated by us to low levels of NO metabolites (NO$_x$) and IL-8 could be evidence of the endothelial cell

dysfunction in patients with LS on their admission of the hospital. In considered case, there was the decrease in both the production and bioavailability of NO in endothelial cells as well the release of IL-8 that could be also produced by endothelial cell as shown in the work by Rakesh et al. [55]. NO is a well-known important factor of vasodilatation and vasoconstriction [48]. IL-8 influences survival and proliferation of endothelial cells and regulates angiogenesis [20,56]. According to our data, the 10-day standard therapeutic course improves the NO_x and IL-8 levels of LS patients. On the other side, at the discharge from the hospital, LS patients had high levels of IL-6 and TNF-α that resulted in high value of coefficient k_R characterizing the systemic inflammation degree. The state of TIA and CHC patients can be associated with the endothelial activation, inflammation and oxidative stress in tissues of the brain [46]. That is proved by high CRP, IL-6 levels and the value of k_R. Moreover, TIA patient state differs from CHC patient state due to the difference in the origin of cerebrovascular accidents. TIA is related to ischemic pathology that makes TIA similar to LS in some extent.

5. CONCLUSION

Thus, our data have shown an important role a number of inflammation markers (NO_x, IL-6, IL-8, TNF-α, CRP) in the pathogenesis of different stroke episodes. Absence of specific neuroimaging changes in TIA and CHC group and nonzero coefficient of reactivity (k_R) suggests that systemic inflammation is not a result of the focal brain ischemia but its intrinsic cause. The differences in the concentration of IL-8, and NO_x in the blood plasma of patients with CHC and LS on their admission to the hospital will help to identify sanogenetic reserves in the case of PIBBC. The observed increase of the TNF-α and IL-6 levels in the plasma of LS patients after the course of standard therapy revealed a new side of the problem.

CONSENT

All authors declare that the written consent was obtained from the patients and the persons included in control group for publication of this case report and accompanying images.

ETHICAL APPROVAL

The study was approved by the Ethics committee of Gomel State Medical University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table 1. The concentrations of IL-6, CRP, IL-8 and NO$_x$ in the blood plasma in patients with LS, TIA, CHC and control subjects group (C) on admission in (index 1, axis x) and on discharge from (index 2, axis y) the stroke unit. The data for each axis are represented as Mdn and the lower (-) and upper (+) limits of IQR

For figure A-D						
For figure A						
	[IL-6]1	**[IL -6]2**	**Δx-**	**Δx+**	**Δy-**	**Δy+**
C	0	0	0	1.3	0	1.3
LS	1.58	5.08	0.7	1	4.9	4.25
TIA	0.81	0.49	0.73	1.05	0.49	1.85
CHC	0.7	0.89	0.46	1.43	0.65	2.44
For figure B						
	[CRP]1	**[CRP]2**	**Δx-**	**Δx+**	**Δy-**	**Δy+**
C	6.4	6.4	3.2	8.3	3.2	8.3
LS	16	21.6	6.31	40.2	5.9	123.2
TIA	14.98	17.4	6.5	55.2	9.6	49.6
CHC	13.1	10.53	6.2	30	5.7	34.6
For figure C						
	[IL -8]1	**[IL -8]2**	**Δx-**	**Δx+**	**Δy-**	**Δy+**
C	5.1	5.1	0.49	1.57	0.49	1.57
LS	3.53	5.75	2.1	0.42	3.69	0.53
TIA	5.81	6.04	1.06	2.77	2.92	1.56
CHC	5.81	7.07	0.35	0.69	0.65	4.02
For figure D						
	[NO$_x$]1	**[NO$_x$]2**	**Δx-**	**Δx+**	**Δy-**	**Δy+**
C	20.35	20.35	6.93	10.17	6.83	10.17
LS	14.56	22.14	5.1	5.92	4.37	27.82
TIA	20.52	26.92	4.96	9.38	11.5	13.81
CHC	24.53	21.85	9.06	8.83	5.03	8.71

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