The Pharma Innovation Journal 2019; 8(2): 118-122

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(2): 118-122

www.thepharmajournal.com
Received: 23-12-2018
Accepted: 28-01-2019

Iryna Ben
Research Institute of Epidemiology and Hygiene, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Correspondence
Iryna Ben
Research Institute of Epidemiology and Hygiene, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Features of clinical manifestations of human granulocytic anaplasmosis in the Western region of Ukraine

Iryna Ben

Abstract
Introduction: Human granulocytic anaplasmosis (HGA) is a transmissive natural focal disease, which is clinically manifested by acute fever, damage of skin, liver, kidney, the nervous system and hematologic disorders. The purpose of our study was to characterize the regional features of clinical manifestations of human granulocytic anaplasmosis, to reveal the most informative clinical and laboratory signs for the diagnosis of HGA, and co-infection HGA with Lyme disease (LD).
Material: We have examined 498 patients with a suspicion of tick-borne diseases with different clinical manifestations who were on treatment in 2006-2014 in regional infectious diseases hospitals in Lviv and Lutsk in Western Ukraine. For confirm HGA diagnosis, the serum of all patients was tested by ELISA for the presence of an antibody (Ig G) to the HGA agent in diagnostic titles (> 100). At the second stage of confirmation of the diagnosis, we determined the *Anaplasma sp.* DNA in the blood by PCR.
Results: Cases of human granulocytic anaplasmosis during 2006-2014 were laboratory verified in 60 patients (12.0 ± 1.5) %. Among the 60 laboratory-verified cases of HGA, only 30 (50 ± 6.5)% were detected with monoinfection, cases co-infection HGA-LD were in 28 ((46.7 ± 6.4) %) patients and patients with antibodies to HGA and TBE were detected in (3.3 ± 0.7) % cases. The main clinical manifestations of HGA monoinfection in the western region of Ukraine are systemic intoxication syndrome (85.7%), hepatitis (50%), acute nephropathy (46.4%), changes in the hemogram (leukopenia with neutropenia, thrombocytopenia) in 85.7% of patients.
Conclusions: The cases of human granulocytic anaplasmosis on the western region of Ukraine were detected and laboratory confirmed. The most characteristic manifestations of HGA monoinfection and co-infection HGA-LD were determined.

Keywords: Human granulocytic anaplasmosis, symptoms, *Anaplasma phagocytophilum*, Lyme disease, tick-borne infections, Ukraine

Introduction
Human granulocytic anaplasmosis (HGA) is a transmissive natural focal disease, which is clinically manifested by acute fever, damage of skin, liver, kidney, the nervous system and hematologic disorders [1].
The causative agent of the infection is *Anaplasma phagocytophilum (A. phagocytophilla)* of the genus *Anaplasma* of the Anaplasmataceae family [2]. The disease is characterized by a wide range of clinical manifestations and a high risk of complications [3]. Mortality in Europe is 1-3%, and reaches 7-10% in the United States [4].
The urgency of the problem of human granulocytic anaplasmosis in Europe and North America is determined by economic losses due to the high incidence of the disease and the widespread occurrence. It occupies the third place after Lyme disease (LD) and tick-borne encephalitis (TBE) among zoonotic diseases [5] in many European countries, including − bordering on Ukraine [6]. In Ukraine, the theoretical and practical aspects of this infectious disease are studied, therefore, practitioners need more information about this illness.
The purpose of our study was to characterize the regional features of clinical manifestations of human granulocytic anaplasmosis, to reveal the most informative clinical and laboratory signs for the diagnosis of HGA, and co-infection HGA with LD.

Material and Methods.
We have examined 498 patients with a suspicion of tick-borne diseases with different clinical manifestations who were on treatment in 2006-2014 in regional infectious diseases hospitals in Lviv and Lutsk in Western Ukraine.

Criteria for inclusion in study based on the presence of the patient's main manifestations after tick bite:

- hyperthermia and the effects of intoxication - weakness, fatigue, headache, myalgia;
- skin lesions - a spotty rash of pink color on the limbs, which later extended to the body and face;
- signs of liver damage - liver enlargement, scleral icteriosis, increase in liver enzymes (transaminases);
- changes in the blood analysis - leukopenia, thrombocytopenia;
- epidemiological criteria - tick bite or stay on the endemic territory with naturally-focal infections (in the forest) etc.

Exclusion criteria are the presence of antibodies to viral hepatitis B and C.

For confirm HGA diagnosis, the serum of all patients was tested by ELISA for the presence of an antibody (Ig G) to the HGA agent in diagnostic titles (>100),with commercial test systems manufactured by Omnix® (St. Petersburg, Russia). The ELISA determination was carried out in accordance with the instructions of the manufacturers of test systems using the control sera (K +, K-) in the solid phase on the 96-well polystyrene tablets using Engvall *et al*. [7]. The reaction results were taken into account using the Biotek immuno-enzymatic photoelectric analyzers at a wavelength of 450 nm.

At the second stage of confirmation of the diagnosis, we determined the *Anaplasma sp*. DNA in the blood. PCR for detection used the commercial diagnostic test system Ampli-HGA®, while Omnix® (St. Petersburg, Russia) Real-time PCR results were recorded using the Rotor-Gene TM 6000 amplifier and the Thermal Cycler System software.

Taking into account a significant proportion of tick-borne co-infections among 498 patients who were examined at HGA, some of them were examined at the same time on LD (452), on TBE (211).

The detection of Ig G antibodies to Lyme disease agent was carried out by the ELISA method using commercial test systems manufactured by Omnix® (St. Petersburg, Russia). The reaction results were taken into account using the Biotek immuno-enzymatic photoelectric analyzers at a wavelength of 450 nm.

To determine the Ig M antibodies to the TBE virus, a test system for the production of Vector-Best ® (Russia) was used. The reaction results were taken into account using the Biotek immuno-enzymatic photoelectric analyzers at a wavelength of 450 nm.

The statistical processing of the obtained results was performed with the program package "Statistica 6.1", using non-parametric (Mann-Whitney) and parametric (Spirman) methods for evaluating the obtained results.

Results

Cases of human granulocytic anaplasmosis during 2006-2014 were laboratory verified in 60 patients (12.0 ± 1.5) % − residents of 10 cities and 15 administrative districts of Volyn and Lviv oblasts.

The group included 31 male and 29 female, the male to female ratio was 1.1: 1. The average age of patients with HGA was 49.2 ± 2.88 years, the age range ranged from 16 to 72 years. It was found that 2/3 (66.7 ± 6.1) % of all patients in this sample comprised individuals aged 31 to 60 years.

Among the 60 laboratory-verified cases of HGA, only 30 (50 ± 6.5) % were detected with monoinfection, cases co-infection HGA-LD were in 28 ((46.7 ± 6.4) %) patients and patients with antibodies to HGA and TBE were detected in (3.3 ± 0.7) % cases.

The fever lasted from 3 to 10 days (an average of 3.9 ± 0.29 days). An increase in body temperature was observed in 17 (56.7%) patients. Rash were found in more than half of the patients − 16 (53.3%) and weakness −13 (43.3%) patients, which were combined with headache − 10 (33.3%) patients, myalgia − 7 (23.3%) and arthralgia of large joints − 4 (13.3%) patients.

Two (7.1%) patients had a pale pink spotty rash on the trunk and limbs, which was not accompanied by any subjective sensations of pain or itching.

Among patients with monoinfection HGA, 14 (50%) patients had liver damage: 7 (25.0%) of them had icteric sclera and skin, 4 (14.3%) patients complained of dark urine. At further examination in 13 (46.4%) of them the liver enlargement was detected.

Taking into account the prevalence of liver disease in the population, the results of laboratory tests, namely, biochemical parameters of blood in patients with monoinfection HGA were compared with the data of biochemical blood tests of healthy persons, candidates for blood donors (n = 50), which did not differ significantly in age from the group of patients with HGA (Table 1).

Table 1: Comparison of indicators of biochemical blood analysis in patients with HGA and healthy individuals, candidates for blood donors

Biochemical indicators	Patients with HGA (n=30)		Healthy individuals, candidates for blood donors (n=50)		p
	Abs. number	M (%)	Abs. number	M (%)	
High bilirubin level	11	36.7	1	2	<0.001*
High transaminases level	11	36.7	4	8	<0.01
High alkaline phosphatase level	10	33.3	1	2	<0.001*

*p<0.05 – a significant difference between the indicators in patients with HGA and healthy individuals

High bilirubin level was detected in 11 (36.7%) patients, compared to control group − only 2% (p<0.001). The highest level of total bilirubin in the blood of the patient with HGA was 48.73 μmol/L. A similar dependence was established in the study of the activity of transaminases, in 11 patients (36.7%) high transaminases level was detected, in contrast to 8% in the control group (p<0.01). An increase in alkaline phosphatase was found in 10 (33.3%) patients, which is higher than the corresponding indicators among healthy donors — 2% (p<0.001).

Kidney damage is one of the most important manifestations of human granulocytic anaplasmosis. In 5 (17.9%) patients with HGA, we found an increase in urea (8.3-9.4 mmol/l) and creatinine (120-138 mmol/l), indicating the presence of nephropathy in the acute period. In 3 (10.7%) patients, the presence of protein was determined in the urine, and 10 (35.7%) patients had epithelial cells (>10) in urine.

The following changes were detected in the hemogram of HGA patients (Table 2): anemia was detected in 2 patients (6.7%) − Hb 105 and 107 g/l. The confirmation of the specific effect of *A. phagocytophilum* on blood cells was a change in the leukocyte formula in all patients with HGA. Most

commonly in blood analysis were detected: leukopenia in 21 (70.0%) patients and lymphopenia in 18 (60.0%) patients, while leukocytosis was found in only 4 (13.3%) patients, an increase in ESR in 30.0% of patients.

Table 2: Indicators of blood analysis in patients with HGA and healthy individuals, candidates for blood donors

Indicators of blood analysis	Patients with HGA (n=30)		Healthy individuals, candidates for blood donors (n=50)		p
	Abs. number	M (%)	Abs. number	M (%)	
Anemia	2	6.7	1	2	>0,1
Leukopenia	21	70.0	1	2	<0,001*
Leukocytosis	4	13.3	2	4	>0,1
Lymphopenia	18	60.0	1	2	<0,001*
Thrombocytopenia	2	6.7	-	-	
Increasing the ESR	9	30.0	1	2	<0,01

*p<0.05 – a significant difference between the indicators in patients with HGA and healthy individuals

As the most important feature of HGA in the western region of Ukraine is a significant proportion of co-infections, which is confirmed by the existence of the combined foci of HGA and LD, our next task was to determine the clinical manifestations in patients with co-infection with LD. Among 28 (46.7±6.4%) cases of co-infection, HGA-LD: 21 (35.0±6.1%) of them were diagnosed co- infection HGA with LD in the stage of localized infection and at 7 (11.7±4.1%) − with LD in the disseminated stage.

The most frequent manifestation of HGA and LD in the stage of localized infection was identified by migratory erythema, which was detected in 18 (85.7%) patients. Its diameter averaged 19.3 cm and varied from 7 to 34 cm. Erythema occurred at the site of tick bite and predominantly localized on the legs − in 10 of 18 patients, which was 55.6%.

Manifestations of systemic intoxication syndrome were detected in 13 (61.9%) patients, but the acute onset of the disease with a sudden increase of body temperature to the febrile level (+ 38 ° C and>) was observed only in 6 (28.6%) patients. In 5 (23.8%) patients, the disease began with an increase of body temperature to the subfebrile level, followed by erythema on 2-4 days.

Damage to the nervous system was detected in 5 (23.8%) patients with co-infection HGA and LD in the localized stage. The development of neurological lesions were in the period from 5 to 35 days and the average was 16.4 days from tick bite.

A comparative study of clinical manifestations of co-infection HGA and LD in the stage of localized infection and HGA monoinfection of revealed a number of differences (Table 3).

Table 3: Analysis of clinical manifestations of HGA monoinfection and co-infection HGA and LD in the stage of localized infection

№	Clinical Signs	Frequency				p
		HGA monoinfection (30)		co-infection HGA and LD (21)		
		Abs. number	M±m (%)	Abs. number	M±m (%)	
1.	Migratory erythema	0		18	85.7±4.9	<0,05*
2.	Icteric sclera and skin	7	23.3 ±5.9	0		<0,05*
3.	Liver enlargement	13	43.3±6.9	7	33.3±6.6	>0,05
4.	High bilirubin level	11	36.3±6.7	2	9.5±4.1	<0,02
5.	High transaminases level	11	36.3±6.7	2	9.5±4.1	<0,02
6.	Leukocytosis	4	13.3±4.8	10	47.6±7.0	<0,01
7.	Leukopenia	21	70.0±6.4	4	19.0±5.5	<0,001*

*p<0.05 – a significant difference between the indicators in patients with HGA and patients with HGA-LD

Liver enlargement was observed in 7 (33.3%) patients with co-infection HGA and LD and in 13 (43.3%) patients with HGA monoinfected (p> 0.05). In cases of co-infection with HGA and LD, a slight increase of bilirubin and transaminases level was observed in 2 (9.5%) patients, significantly lower, than in HGA monoinfection − 11 (36.3%) patients indicated an increase in bilirubin and transaminases level and in 7 (23.3%) patients had icteric sclera and skin (p<0.02).

Renal impairment in co-infection HGA and LD was not observed, unlike HGA monoinfection, in which renal involvement was manifested in 5 (16.7%) patients with elevated urea (8.3-9.4 mmol/L) and creatinine (120-138 mmol/L).

In the case of co-infection HGA and LD in the hemogram was observed leukopenia in 4 (19.0%) and leukocytosis in 10 (47.6%) patients. Leukopenia was detected in 21 (70.0%) and much less (p<0.001) leukocytosis in 4 (13.3%) patients with HGA monoinfection (p<0.01).

The cases of co-infection HGA with LD in the stage of dissemination were more difficult. Among 7 patients with co-infection HGA-LD in the stage of dissemination, the signs of systemic intoxication syndrome were noted in 3 (42.3%). The lesions of the nervous system in the form of cerebrospinal symptoms (headache, dizziness, paresthesia and tremor of the upper limbs) occurred in 3 (42.3%) patients, cardiovascular disorders (relative bradycardia, palpitation) and respiratory system (pharyngitis) − in 2 (28.6%) patients, musculoskeletal system (arthritis and synovitis) and liver − in 2 (28.6%) patients, respectively.

Discussion

For HGA monoinfection were characterized by an acute course with the prevalence of febrile fever and severe systemic intoxication syndrome, which corresponds to the literature [8]. The rash belongs to typical and important manifestations [9, 10], which, according to various authors, is resorted with a frequency of 1% [8] to 16% [10], and our patients are found to be 7.1%.

Liver impairment of HGA, according to the literature [4, 8-12], are among the most important manifestations and occur in 83-85.1% of patients. However, in our patients, elevated bilirubin level and high transaminases level were detected in 11 (39.3%) patients, while elevated alkaline phosphatase and thyme samples were detected in 10 (35.7%) patients, which

can be attributed to the HGA characteristics of the Western region.

The study of the manifestations spectrum in patients with HGA cases and a comparative analysis of the data obtained by other researchers [8-11] and we showed that the frequency of lesions of individual organs and systems of the body of patients in the western region of Ukraine had certain features. The distinctive features of laboratory confirmed cases of HGA in our studies include a higher percentage of leukopenia (71.4%) than in Europe (47%), as well as a lower index of kidney damage (17.9%) than in Russia (29.8%), respectively [13, 14].

The clinical data we obtained about the course of co-infection HGA and LD in the stage of localized infection coincided with the results of studies of foreign authors regarding the high frequency ME [8, 10]. Particular attention deserves the study of clinical signs of liver impairment. According to Russian authors who describe liver enlargement from 33% [15] to 63.6% [16] with HGA-LD co-infection, we observed an increase in liver in only 7 (33.3%) patients. An increase of bilirubin level was determined in individual patients and according to our data and literature data. However, the growth of transaminases level was established by us only in 2 (9.5%) patients, as opposed to 41.5%, according to other authors [15, 17, 18].

Changes in the hemogram, such as leukopenia and leukocytosis in HGA-LD co-infection were observed in 4 (19.0%) and 10 (47.6%) patients respectively. In contrast to the literature [15, 17, 18], where leukopenia was detected in 13.3-51.9% of patients, and leukocytosis in 3.9% of patients [15, 18].

The results of the clinical manifestations analyzed of laboratory-confirmed cases of co-infection HGA with the disseminated form of LD coincide with the data of studies of foreign authors [1, 4, 15, 17-19].

Conclusions

The cases of human granulocytic anaplasmosis on the territory of the western region of Ukraine were detected and laboratory confirmed.

It was established that HGA monoinfection is (50 ± 6.5) %, co-infection HGA and Lyme disease − (46.7±6.4) %, the co-infection HGA and TBE − (3.3±0,7) %.

The main clinical manifestations of HGA monoinfection in the western region of Ukraine are systemic intoxication syndrome (85.7%), hepatitis (50%), acute nephropathy (46.4%), changes in the hemogram (leukopenia with neutropenia, thrombocytopenia) in 85.7% of patients.

The most frequent manifestation of co-infection HGA-LD in the stage of localized infection is migratory erythema, which was detected in 18 (85.7%) patients.

A combination of human granulocytic anaplasmosis with a disseminated form of Lyme disease has a severe course with lesions of the nervous system, musculoskeletal system and cardiovascular system.

Availability of data and materials: Data are available upon request to the first author.

Competing interests. There are no competing interests.

Funding: Research Institute of Epidemiology and Hygiene Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

References

1. Bakken JS, Dumler JS. Human granulocytic anaplasmosis. Infect Dis Clin North Am. 2015; 29(2):341-355.
2. Atif FA. Alpha proteobacteria of genus *Anaplasma* (Rickettsiales: Anaplasmataceae): Epidemiology and characteristics of *Anaplasma* species related to veterinary and public health importance. Parasitology. 2016; 143(6):659-685.
3. Dumler JS. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. FEMS Immunol Med Microbiol. 2012; 64(1):13-20.
4. Weil AA, Baron EL, Brown CM, Drapkin MS. Clinical findings and diagnosis in human granulocytic anaplasmosis: A case series from Massachusetts. Mayo Clin Proc. 2012; 87(3):233-239.
5. Dugat Th, Lagrée AC, Maillard R, Boulouis HJ, Haddad N. Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. Front Cell Infect Microbiol. 2015; 5(61):1-18.
6. Kmieciak W, Ciszewski M, Szewczyk EM. Tick-borne diseases in Poland: Prevalence and difficulties in diagnostics. Medycyna pracy. 2016; 67(1):73-87.
7. Engvall E, Perlmann P. Ensime-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochem. 1971; 8:871-874.
8. Afanaseva MV, Vorobeva NN, Korenberg EI. Granulotsitarnyiy anaplazmoz cheloveka: osobennosti klinicheskih proyavleniy v Rossii. Infektsionnyie bolezni [in Russian]. 2006; 4(2):24-28.
9. Clair KSt, Decker CF. Ehrlichioses: anaplasmosis and human ehrlichiosis. Dis Mon. 2012; 58(6):346-354.
10. Sanchez E, Vannier E, Wormser GP, Hu LT. Diagnosis, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: A Review. JAMA. 2016; 315(16):1767-1777.
11. Mayne PJ. Clinical determinants of Lyme borreliosis, babesiosis, bartonellosis, anaplasmosis, and ehrlichiosis in an Australian cohort. Int J Gen Med. 2014; 8:15-26.
12. Dahlgren FS, Heitman KN, Drexler NA, Massung RF, Behravesh CB. Human granulocytic anaplasmosis in the United States from 2008 to 2012: a summary of national surveillance data. Am J Trop Med Hyg. 2015; 93(1):66-72.
13. Dumler JS, Bakken JS. Human granulocytic ehrlichiosis in Wisconsin and Minnesota: a frequent infection with the potential for persistence. J Infect Dis. 1996; 173:1027-1030.
14. Kernif T, Leulmi H, Raoult D, Parola P. Emerging Tick-Borne Bacterial Pathogens. Microbiol Spectr. 2016; 4(3):1128.
15. Aleshkovskaya ES, Blagov NA, Bazunova VA. Kliniko-epidemiologicheskie aspektyi smeshannyih kleschevyih infektsiy v endemichnom regione. Zhurnal infektologii [in Russian]. 2013; 5(1):44-47.
16. Koebel C, Kern A, Edouard S, Hoang AT, Celestin N, Hansmann Y *et al*. Human granulocytic anaplasmosis in eastern France: clinical presentation and laboratory diagnosis. Diagn Microbiol Infect Dis. 2012; 72(3):214-218.
17. Teterin VYu, Korenberg EI, Nefedova VV. Granulotsitarnyiy anaplazmoz cheloveka i mikst-infektsiya s iksodovyim kleschevyim borreliozom.

Infektsionnyie bolezni [in Russian]. 2012; 10(1):21-27.
18. Horowitz HW, Aguero-Rosenfeld ME, Holmgren D, McKenna D, Schwartz I, Cox ME *et al*. Lyme Disease and Human Granulocytic Anaplasmosis Coinfection: Impact of Case Definition on Coinfection Rates and Illness Severity. Clin Infect Dis. 2012; 11:154-161.
19. Berghoff W. Chronic Lyme disease and Co-infections: Differential Diagnosis. Open Neurol J. 2012; 6:158-178.