people without clinical history of loxoscelism were part of the control group (Group 2).

Group 1 was defined based on:

- loxoscelism diagnosis according to a clinical history;
- a patient's own assertion of having been bitten by some arachnid (association with biting by *L. laeta*) with and without dermonecrotic or visceral loxoscelism;
- visual assessment of current or previous dermonecrotic lesions. People who reported being bitten by an arachnid were identified and confirmed for loxoscelism dermonecrotic lesions at the Molecular Parasitology Laboratory of the Faculty of Health Sciences of the Universidad de Antofagasta, according to the clinical guide for handling the bites of the corner spider from the Chilean Ministry of Health [24].

People in Group 2 were defined based on:

- no verifiable clinical history of loxoscelism or having previously suffered a bite from *L. laeta* or other type of arachnid;
- no presentation of chronic diseases or allergies;
- no evidence of autoimmune diseases, rheumatoid arthritis, or any known physical illness affecting their immunological status;
- no acute infectious processes at the time of sample extraction; and
- no presence of skin lesions attributable to infectious bacterial processes.

Among the samples in Group 1, 60% were from patients with cutaneous loxoscelism and 40% were from patients with viscero-cutaneous loxoscelism. The age range of people of groups 1 and 2 was 18 to 60 years old, and both groups had equal proportions of men and women (Table 1).

Spider venom, recombinant phospholipase D expression and purification

The recombinant protein rLlPLD1 was expressed and purified as previously described [25]. In addition, the nucleotide sequences for different phospholipase D isoforms of the four representative species of *Loxosceles* (*L. laeta*, *L. intermedia*, *L. reclusa* and *L. gaucho*) available in GeneBank (NCBI) were used for expression of their ORFs in *E. coli* BL21 DE3 and purified as fusion proteins with a 6His tag at the N-terminus (LrSMD1 and LgDerProt1) or at the C-terminus (rLlPLD2 and LiDerTox1) by GenScript (GenScript Inc., USA). GenBank accession numbers for nucleotide sequences used and the molecular masses of the respective recombinant proteins were: *L. laeta* PLD isoform 2 (LlPLD2), access n° GU121906 [25], 32,055 Da with C-His tag; *L. reclusa* sphingomyelinase D isoform 1 (LrSMD1), access n° AY559846.1 [26], 31,219 Da with N-His tag; *L. intermedia* sphingomyelinase P1 (LiSMD P1), access n° AY304471.2 [27], 34,982 Da and C-His tag; and *L. gaucho* dermonecrotic protein 1 (LgDerProt1), access n° AY974250.1, 31,172 Da with N-His tag. Moreover, venom from 20 female *L. laeta* and 20 *Sicarius* adults was extracted by electrostimulation and collected as previously reported [28]. Polyclonal mouse anti-*L. laeta* venom antibodies were prepared as previously documented [25].

Table 1 Distribution of serum samples from patients groups with and without loxoscelism

Age group	Without loxoscelism[a] ($n = 30$)		With loxoscelism[a]			
			Cutaneous[b] ($n = 6$)		Systemic[b] ($n = 4$)	
	Male	Female	Male	Female	Male	Female
18–29	11/36.7%	16/53.3%	1/16.7%	2/33.3%	2/50%	1/25%
30–60	3/10%	–	2/33.3%	1/16.7%	–	1/25%

[a]Samples of serum obtained under informed consent (procedure approved by the Research Ethics Committee of the Universidad de Antofagasta – CEIC-UA)
[b]According to clinical history and/or direct observation of dermonecrotic lesion. Samples are represented as number and percentage (n/%)

Dot blot to determine *L. laeta* antivenom antibodies

Dot blot for *L. laeta* venom antibody detection was assembled in our laboratory, with 1 μg of *L. laeta* venom being adsorbed onto a nitrocellulose membrane using a 96-well Dot-Blot Filtration Manifold System (Gibco BRL). The presence of adsorbed proteins on the membrane was evaluated by staining with Ponceau red. The membrane was then blocked for 1 h at 22–25 °C with 5% non-fat milk in PBS/0.1% Tween20 (PBS-T). Each dot was then incubated with a pool of Group 1 or Group 2 sera (1:1000 dilution), and alternatively with single serums of Group 1 or Group 2 at a 1:10 dilution. The membranes were washed three times with PBS-T and incubated for 1 h at 22–25 °C with the anti-human IgG-HRP secondary antibody in 1:50,000 dilution, then washed again three times with PBS-T and developed by ECL.

PBS or pre-immune mouse serum (1:1000 dilution) was used as the negative control. As positive control, mouse anti-*L. laeta* venom serum (1:10,000 dilution) and monoclonal antibody 7E4-D2 anti-rL1PLD1 (1:50,000 dilution) were used [25]. BSA was used as unrelated antigen to evaluate specificity of reaction. Images were captured on a ChemiBis 2.0 DNR photo-documenter (DNR Bio-Imaging Systems Ltd., Jerusalem, Israel). Intensity of dots was evaluated by densitometry and expressed as relative density percentage.

Indirect ELISA for detection of *L. laeta* venom antibodies

The titration of different sera was carried out using an indirect ELISA for detecting specific circulating antibodies against *L. laeta* venom, based on previously published protocols [29], and mounted in our laboratory. On 96-well