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Kaustuv Nayak, ... , Pratima Ray, Kaja Murali-Krishna

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Chikungunya virus (CHIKV) infection causes acute febrile illness in humans and some of these individuals develop a debilitating chronic arthritis that can persist for months to years for reasons that remain poorly understood. In this study from India, we characterized antibody response patterns in chikungunya febrile patients and further assessed the association of these initial febrile phase antibody response patterns with protection versus progression to developing chronic arthritis. We found five distinct patterns of the antibody responses in febrile phase: No CHIKV binding or Neutralizing (NT) antibodies but PCR positive, IgM alone with no NT activity, IgM alone with NT activity, IgM and IgG without NT activity, IgM and IgG with NT activity. A 20-month follow-up showed that appearance of NT activity regardless of antibody isotype or appearance of IgG regardless of NT activity during the initial febrile phase is associated with a robust protection against developing chronic arthritis in the future. These findings, while providing novel insights on correlates of protective immunity against chikungunya-induced chronic arthritis, suggest that qualitative differences in the antibody response patterns that have evolved during the febrile phase can serve as biomarkers, that allow prediction of protection or progression to chronic arthritis in the future.

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## **Antibody response patterns in chikungunya febrile phase predicts protection versus progression to chronic arthritis**

Kaustuv Nayak\*<sup>1</sup>, Vineet Jain\*<sup>2</sup>, Manpreet Kaur<sup>1</sup>, Naushad Khan<sup>3</sup>, Kamalvishnu Gottimukkala<sup>1</sup>, Charu Aggarwal<sup>1</sup>, Rohit Sagar<sup>3</sup>, Shipra Gupta<sup>3</sup>, Ramesh Chandra Rai<sup>1</sup>, Kritika Dixit<sup>1</sup>, Mohammad Islamuddin<sup>3</sup>, Anil Verma<sup>4</sup>, Deepti Maheshwari<sup>1</sup>, Yadya M.Chawla<sup>1</sup>, Elluri Seetharami Reddy<sup>1</sup>, Harekrushna Panda<sup>1</sup>, Pragati Sharma<sup>1</sup>, Priya Bhatnagar<sup>1</sup>, Prabhat Singh<sup>1</sup>, Siva Raghavendhar B<sup>5</sup>, Ashok Kumar Patel<sup>5</sup>, Vinod H Ratageri<sup>6</sup>, Anmol Chandele<sup>1</sup>, Pratima Ray<sup>#3</sup>, Kaja Murali Krishna <sup>#1, 7,8</sup>

<sup>1</sup> ICGEB-Emory Vaccine Center, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India

<sup>2</sup> Department of Medicine, Hamdard Institute of Medical Sciences and Research (HIMSAR), Jamia Hamdard, New Delhi-110062, India

<sup>3</sup> Department of Biotechnology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi – 110062, India

<sup>4</sup> Department of Pediatrics, All India Institute of Medical Sciences, New Delhi-110029, India.

<sup>5</sup> Kusuma School of Biological Sciences, Indian Institute of Technology (IIT), New Delhi, India

<sup>6</sup> Department of Pediatrics, Karnataka Institute of Medical Sciences (KIMS), Hubli-580032, Karnataka, India.

<sup>7</sup> Emory Vaccine Center, Emory University School of Medicine, Atlanta, Georgia-30322, USA

<sup>8</sup> Department of Pediatrics, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia-30322, USA

\* Co-First Authors

#Co-Senior Authors

### **Corresponding Address:**

Murali Krishna Kaja  
Department of Pediatrics, Division of Infectious Diseases,  
Emory University School of Medicine,  
Atlanta, GA 30322  
+1-404-858-7458  
murali.kaja@emory.edu

### **Conflict of Interest statement:**

The authors have declared that no conflict of interest exists.

## **Abstract**

Chikungunya virus (CHIKV) infection causes acute febrile illness in humans and some of these individuals develop a debilitating chronic arthritis that can persist for months to years for reasons that remain poorly understood. In this study from India, we characterized antibody response patterns in chikungunya febrile patients and further assessed the association of these initial febrile phase antibody response patterns with protection versus progression to developing chronic arthritis. We found five distinct patterns of the antibody responses in febrile phase: No CHIKV binding or Neutralizing (NT) antibodies but PCR positive, IgM alone with no NT activity, IgM alone with NT activity, IgM and IgG without NT activity, IgM and IgG with NT activity. A 20-month follow-up showed that appearance of NT activity regardless of antibody isotype or appearance of IgG regardless of NT activity during the initial febrile phase is associated with a robust protection against developing chronic arthritis in the future. These findings, while providing novel insights on correlates of protective immunity against chikungunya-induced chronic arthritis, suggest that qualitative differences in the antibody response patterns that have evolved during the febrile phase can serve as biomarkers, that allow prediction of protection or progression to chronic arthritis in the future.

## **Introduction**

Chikungunya is emerging as an important mosquito borne arboviral disease of global importance to human health (1-4). Although originally discovered in Africa in 1952, large outbreaks of chikungunya virus (CHIKV) started appearing from 2005, first in Réunion Island (5), and then quickly in India (6-11) followed by a rapid spread to over 40 countries in South East Asia, Caribbean, Central Europe and more recently to the Americas (12). Since the major outbreak that occurred in India in 2006, CHIKV has quickly spread throughout the country and now affects millions of people each year (8). While a vast majority of CHIKV infected naïve individuals develop a febrile illness with joint pain (arthralgia) and/or joint swelling (arthritis), some, but not all, of these affected individuals develop a debilitating chronic arthritis (13-15). The proportion of the affected individuals that develop chronic arthritis is estimated to vary between 10-50% depending upon the study design and the duration of the follow up. While many studies have indicated high viral load (16) or inflammatory mediators (17) are associated with the severity of the disease during the febrile phase, what immune factors that are induced during the acute febrile phase predict protection or progression to chronic arthritis, and its duration, remains poorly understood. Previous studies from our and other groups indicated that an evolution of antibody responses with neutralizing activity early after onset of chronic disease is important for protection (18, 19) What antibody profiles are induced during the febrile phase itself and how do they associate with protection versus progression to developing chronic arthritis in the future, especially in a long-term follow up, remained less clear.

In this study, we assessed the diversity of the antibody response patterns in chikungunya confirmed acute febrile patients from India and then evaluated the impact of these febrile antibody response patterns on the downstream outcome of protection versus progression to chronic arthritis in a 20-month follow up.

## Results

### ***CHIKV patients show five distinct antibody response patterns during the febrile phase.***

We recruited a total of 434 patients with chikungunya suspected symptoms during CHIKV transmission season from 2014-2016. Recruitment strategy is outlined in **Figure S1**. Of these, 184 patients were confirmed as CHIKV cases based on plasma positivity to CHIKV PCR and/or IgM. Among these CHIKV confirmed, 133 were acute febrile cases (fever < 10 days), and 51 were early or late chronic CHIKV cases (symptoms persisting for 10-40 days post febrile episode). Clinical and demographic characteristics of these chikungunya confirmed febrile and chronic patients are shown in **Table-S1**.

Analysis of CHIKV-specific IgM and IgG in individual patients revealed three broad antibody response patterns that have evolved during the CHIKV acute febrile phase (**Figure 1A, left**); No detectable IgM or IgG antibody but positive for PCR, positive for IgM alone, or positive for both IgM and IgG. Among the patients that had induced both IgM and IgG during the febrile phase, 31.9% patients had IgM > IgG phenotype and 68.1% were IgG > IgM phenotype. Characterization of neutralizing (NT) activity within these three antibody response patterns further expanded them to five distinct groups during the febrile phase (**Figure 1B, left**). These include, Group I, no CHIKV binding or NT antibodies but positive for PCR; Group II, IgM alone without NT activity; Group III, IgM alone with NT activity; Group IV, IgM and IgG without NT activity, Group V, IgM and IgG with NT activity. The relative proportion of patients evolving each of these five distinct antibody patterns in the acute febrile phase is shown in **Figure 1C, left**.

Amongst the isotype switched febrile cases (Group IV and V), a relatively higher proportion of the NT activity positive individuals showed an IgG dominated response (Group V, 76%) compared to the NT activity negative individuals (Group IV, 35%) (**Figure S2**). These diverse antibody response patterns that were observed during the febrile phase became progressively more uniform towards an IgG dominated isotype switched response along with neutralizing activity immediately after the febrile phase during the early chronic phase (**Figure 1A,B,C, middle panel**), and late chronic phases (**Figure 1A,B,C right panels**).

Taken together, these results show that while chronic CHIKV patients show a relatively uniform response of IgM, IgG and NT activity, there is remarkable heterogeneity in the patterns of the antibody responses that evolve during the CHIKV acute febrile phase.

***Appearance of NT activity or isotype switching in the febrile phase was associated with low viral loads.***

We questioned whether these distinct antibody profiles that were observed during the acute febrile phase were simply related to differences in the age of the patient or the day of fever (DOF). Although mean age of the patient (**Figure 2A**) or the average DOF (**Figure 2B**) were strikingly similar among the five groups, individuals that have not developed any antibodies but were PCR positive were at marginally earlier days of fever (**Figure 2B, Group I**); suggesting that the evolution of these antibody response patterns are likely to

be highly dynamic in nature within the febrile phase. Consistent with this, even among the isotype switched individuals, IgG was only moderately higher in Group V patients (that had evolved NT activity) compared to Group IV patients (that were lacking NT activity) (**Figure 2D, Groups IV, V**). Analysis of the IgG subtypes revealed that this IgG response, when induced within the febrile phase (i.e., group IV and group V patients), was comprised of a mix of IgG1, IgG2 and IgG3 isotypes regardless of the evolution of NT activity (**Supplementary Figure S3**). The IgM levels, when induced, were strikingly similar regardless of the NT activity or isotype switch status in the acute febrile phase (**Figure 2C, Groups II, III, IV, V**). Although the NT antibody titers were relatively lower in Group III (that had IgM alone without NT activity) compared to Group V (that had IgM, IgG and NT activity) in the febrile phase (**Figure 2E, Group III, V**), the appearance of the NT activity (group III and V) as well as the occurrence of isotype switching (Group IV and V) seems associated with a better viral control during the febrile phase. This is evidenced by a much lower proportion of the patients in who virus was recoverable in group III, group IV and group V patients (50%, 24%, 22% respectively) compared to group I or group II patients (100% and 72% respectively). (**Table S2**). Consistent with this, the viral load, where recovered, was also found to be lowest in group III, group IV and group V patients (virus copy numbers  $10^5$  or less) compared to Group I or group II patients (virus copy numbers,  $10^8$  or more) (**Figure 2F**). Taken together, these results suggested that appearance of NT activity regardless of isotype switch status or occurrence of isotype switching regardless of NT activity was associated with better viral control in the febrile phase.

***The NT activity in Group III patients was mediated by IgM, whereas the NT activity in Group V patients was mediated by either IgG or a combination of IgG and IgM.***

In the plasma of Group III febrile patients, we found a tight correlation between the NT titers and IgM levels (**Figure 3A, third panel**). *In vitro* destruction of IgM led to a decrease in the NT titers of these plasma (**Figure 3C, left graph**) confirming that IgM primarily mediates the neutralizing activity in this group. On the other hand, in the plasma of Group V patients, although NT antibody titers showed a better correlation with IgG (**Figure 3B, right most**) than with IgM titers (**Figure 3A, right most**), *in vitro* destruction of IgM in these plasma suggested that the NT activity in this group was mediated by IgG in 52% patients (**Figure 3C, right graph, gray lines**), and by a combination of IgM & IgG in 48% of these patients (**Figure 3C, right graph, red lines**). Taken together, these results suggested that, among the patients that have isotype switched and also evolved NT activity, the NT activity was mediated by either IgG or a combination of IgG and IgM.

***Arthritis was observed during the acute febrile phase regardless of the antibody response patterns.***

We then wondered whether arthritis was associated with any specific antibody response pattern during the acute febrile phase. There was no statistically significant difference in the proportion of patients with arthritis in each of the five groups during the acute febrile phase (**Figure 4**); suggesting that the diversity of the antibody response patterns induced

during the febrile phase have little influence on the frequency of the arthritis cases during the febrile phase .

**Appearance of NT activity regardless of antibody isotype or appearance of IgG regardless of NT activity during the febrile phase predicts protection against developing chronic arthritis.**

To determine whether there is any relation between the antibody response patterns that were induced during the initial febrile phase on future outcome, namely protection versus progression to developing chronic arthritis, we followed up these patients to assess for development of chronic arthritis. A total of 72 patients were successfully assessed for up to 20 months post febrile phase. **Figure 5** shows percentage of patients with chronic arthritis over the 20 months follow up period in each of the groups depending on the initial antibody response pattern that they had evolved during the initial febrile phase. Development of chronic arthritis was highest in the Group I and Group II patients. Conversely, the development of chronic arthritis was the least in groups III, IV, and V. Taken together, these observations suggest that appearance of NT activity regardless of antibody isotype or appearance of IgG regardless of NT activity during the acute febrile phase is associated with a better protection against developing chronic arthritis in the future.

## Discussion

Our study provides a comprehensive understanding of the diversity of the humoral response patterns that evolve in chikungunya patients during the acute febrile phase. Additionally, our study provides an understanding on whether these initial febrile antibody response patterns have any correlation with protection versus progression to developing chronic arthritis in the future. We show a remarkable heterogeneity in the antibody response patterns induced during the acute febrile phase. These range from no antibodies, IgM alone without NT activity, IgM alone with NT activity, IgM and IgG without NT activity and IgM and IgG with NT activity. We show that patients that have evolved an IgM response with NT activity or an IgM and IgG response with or without NT activity had a better viral control in the febrile phase. By long-term follow up for over 20 months, we show that the appearance of the NT activity regardless of antibody isotype or appearance of IgG regardless of NT activity during the febrile phase is also associated with a better protection against developing chronic arthritis in the future. On the other hand, patients that failed to evolve neutralizing antibodies, or isotype switched responses during the acute febrile phase (i.e., IgM alone without NT activity) are at the highest risk of developing chronic arthritis in the future. These findings have significance for improving our understanding towards developing biomarkers of prognostic value as well as towards vaccine development and evaluation by providing novel insights into correlates of protective immunity against Chikungunya induced chronic arthritis.

Why symptomatic chikungunya patients that are most likely to be primary infections evolve such heterogeneous antibody response patterns remains unknown (20). This

extraordinary heterogeneity of the antibody responses observed during the febrile phase are not static since these responses become more uniform immediately after febrile phase and in late chronic patients. Additionally, by subsequent sampling of a subset of the patients at 2-years post febrile phase, we confirmed that both IgG as well as NT activity evolve regardless of the initial febrile response pattern (**Figure S4**). We speculate that these differences arise due to inter-individual differences in initial viral inoculum (21), initial viral loads (22), incubation period post viral exposure (23), host genetic factors (24, 25) as well as immune responses (26, 27) and may contribute to this remarkable heterogeneity in the antibody response patterns that evolve during the acute febrile phase.

An understanding of how the antibody response patterns that were induced during the acute febrile phase tip the balance between protection vs pathogenesis is important. A previous study hypothesized that IgM produced during Chikv infection may lead to pathology by molecular mimicry (28). Our study showing highest chronic arthritis in group II patients (that generated IgM alone without NT activity during febrile phase) is consistent with this hypothesis. Additionally our observation that patients who evolved an IgM response with NT activity (Group III), as well as the patients that evolved IgM and IgG response with or without NT activity (Group IV, V) had low viral loads during the acute febrile phase and are also highly protected against developing chronic arthritis in the future suggests that these particular antibody response patterns might help tip the balance from pathology to protection, at least in part via a better viral control during the febrile phase itself.

While NT activity mediated viral control is expected, from our studies, it is interesting to note that an early evolution of IgG even without NT activity (group IV) also contributed to protection. Considering that various IgG subtypes can offer protection in an NT independent way in many viral infections, it is important to know what subtypes are induced in this group. Consistent with our group I pattern, A previous study showed that CHIKV patients that were identified based on PCR positivity largely tend to show no antibody response during the febrile phase(29). Interestingly, this study showed that some, but not all of these patients that were lacking any antibody response during the febrile phase, elicited an isotype switched IgG3 response that was associated with NT activity soon after the febrile phase. However, it currently remains unclear as to what IgG subtypes will be induced if an isotype switched responses occurs within the acute febrile phase itself. We have detected both IgG1, IgG2 as well as IgG3 subtypes in our Groups IV (that showed IgG & IgM without NT activity pattern) and in group V patients (that showed IgG & IgM with NT activity) during the febrile phase (**Figure S3**). Considering that IgG1 is known to have longest half- life and an efficient mediator of ADCC, IgG2 an efficient mediator of phagocytosis whereas both IgG1 and IgG3 robustly activate the complement as well as perform Fc mediated phagocytosis. (30-32) We speculate that these NT independent effector functions of the IgG subtypes might contribute to protection observed in group IV patients in our study.

Although the group III, IV and V patients in our study are highly protected, it is important to note that a small fraction of patients (~10%) from these groups continue to have chronic arthritis even after a year of acute episode. This suggests that even though development of mature isotype switched antibody response with or without NT activity or IgM response with NT activity during the acute febrile period is a strong correlate of protection, a minor fraction of the individuals are likely to escape protection despite evolving these protective antibody response patterns during the febrile phase. Correlation analysis between the NT activity, IgM or IgG magnitude between the individuals who were protected versus those that develop chronic arthritis from groups III, IV and V patients showed there were trends, although statistically insignificant, suggesting a relatively lower NT activity or IgG in group III, IV and V patients that were not protected (**Figure S5 A, B C**). Whether the group III, IV and V individuals that escaped the protection differed in other components of innate or adaptive responses such as innate /inflammatory mediators or T and B cell responses during the febrile phase remains to be addressed.

Interestingly, our results suggest that, in addition to IgG with or without NT activity, the IgM mediated NT activity may have beneficial effect *in vivo*. However, there are two limitations in our study. First, all IgG producing patients (~50% of the febrile patient population) invariably had IgM. Hence, it is difficult to decipher the relative *in vivo* role of each of these isotypes in protection against developing chronic arthritis. Second, although the “IgM alone with NT activity group” provides a unique opportunity to understand the *in vivo* role of IgM in protection against developing arthritis, this group represented only a small fraction (7.5%) of the total chikungunya febrile patient population that has been

studied. As a consequence, we could follow up only a small number of the patients from this “IgM alone with NT activity group”. Studies are needed, perhaps by recruiting large numbers of the patients to identify sufficiently large set that had this “IgM associated NT activity alone” to further solidify this finding.

In summary, our findings suggest that individuals who induce a mature antibody response as evidenced by IgG isotype switching, regardless of the NT activity or individuals that induce NT activity, regardless of isotype switching within the acute febrile phase are less prone to chronic arthritis.

## **Methods:**

### **Patient Recruitment**

Patients were recruited at two sites. Hamdard Institute of Medical Sciences and Research (HIMSAR), Jamia Hamdard, New Delhi, India & Karnataka Institute of Medical Sciences (KIMS), Hubli, Karnataka, India. New Delhi (28.6139°N, 77.2090°E) is located in the northern part of India and Hubli (15.3647° N, 75.1240° E) is located in Southern part of India. Both the areas remain highly affected by Chikungunya for the past 10 years. Both the hospitals have excellent medical care, diagnostics and accessibility to medical records as per the Government of India standards. Both primary and tertiary care patients visit these hospitals.

Chikungunya suspected male and female patients of aged between 15-77 years were recruited in the years 2013-2016 during the chikungunya transmission season, that typically range from July-November. The inclusion criteria followed for recruitment included clinical presentation consistent with chikungunya infection which include abrupt onset of fever anywhere within past 40 days and/or symptoms ranging from myalgia, rash, fever, body pains, joint swelling. Pregnant women, patients who were positive for malaria or common respiratory infections such as active tuberculosis, or patients having pre-existing arthritis for more than 40 days prior to onset of symptoms were excluded. Baseline clinical parameters for disease activity including joint pain (arthralgia), joint swelling (arthritis), rash, cough, restlessness, myalgia, stomach cramps and bleeding were recorded. Most commonly affected joints were wrists, metacarpophalangeal joints, proximal interphalangeal joints, elbows, shoulders, knees, ankles and

metatarsophalangeal joints. A blood specimen drawn at the time of initial clinical evaluation was sent for research studies that run a battery of tests to confirm chikungunya infection or to exclude other febrile infections. Only those patients whose samples were positive for CHIKV PCR and/or IgM, negative for dengue NS1 and/or IgM, and also manifesting acute febrile illness for  $\leq 10$  days were considered as acute febrile CHIKV confirmed patients.

The recruitment criteria are shown in **Figure S1**, and the characteristics of the CHIKV confirmed patients are shown in **Table S1**. CHIKV confirmed acute febrile patients were followed up telephonically over the next two years. The questionnaire was administered and recorded by clinical care staff, blinded to the serological status, and made available to the research team without personally identifiable information, only after study completion to ensure the best possible double blinded analysis and anonymity. The questionnaire was composed of closed questions addressing the course of chronic arthritis manifestations, relapse in condition with/without fever to assess the clinical outcome. The status of their joint swelling and its resolution was enquired in explicit details with regards to joint swelling with pain and difficulty during walking, bending, squatting and other day to day activities. Data was collected on disease status at four time points to a maximum of 20 months in a total of 72 patients.

### **Serology Tests**

CHIKV specific IgM and IgG were detected using Chikungunya IgM capture ELISA kits (Abcam, Cat #: Ab177848) and chikungunya IgG capture ELISA kit (Abcam, Cat #:177835) using manufacturer's recommendations. Samples were screened for dengue

specific IgM and IgG levels using Panbio dengue IgM Capture ELISA (Panbio Cat#01PE20) and Panbio dengue IgG capture ELISA (Panbio Cat#01PE10) as per the manufacturer's instructions. Samples were screened for dengue NS1 antigen using dengue day-1 test kit (J Mitra & Co; IR028050) as per the manufacturer's instructions and elaborated in our previous studies(33, 34).

### **In house ELISA for IgG Subtypes**

IgG subtypes were characterized as described (29) CHIKV coated ELISA plates were incubated with 1: 25 diluted plasma followed by washing and incubation with mouse anti-human IgG1 Fc-HRP (Southern Biotech, Cat no. 9054-05), Mouse anti-human IgG2 Fc-HRP (Southern Biotech, Cat no. 9060-05), Mouse anti-human IgG3 hinge-HRP (Southern Biotech, Cat no. 9210-05 ), Mouse anti-human IgG4 Fc-HRP (Southern Biotech, Cat no. 9200-05) at 1:1000 dilution. ELISA color reaction was developed using TBM substrate (Mabtech, Cat # 3652-F10) and optical density (OD) was measured in ELISA reader at 490 nm wavelength.

### **CHIKV neutralizing antibody assays**

NT activity of plasma samples was evaluated using plaque reduction neutralization assays (PRNT) as described previously (18). Vero cells (ATCC, Cat # CCL-81) were plated on 96-well plates at a concentration of 30,000 cells per well. After overnight culture, the cells were infected with 50 plaque forming units of CHIKV per well that was pre-incubated for 1 hour with graded 2-fold dilutions of the heat inactivated plasma (ranging

from 1:50 to 1:3200). After incubation, the cell layer was overlaid with 2% Carboxymethyl cellulose in Opti-MEM media (Invitrogen Cat # 31985070) with 2% FBS, amphotericin B, ciprofloxacin and incubated for an additional 36 hours. After this, the cell layer was stained using 0.25% crystal violet in 30% methanol and the plaques were counted. The neutralizing titer (PRNT<sub>50</sub>) was expressed as the plasma dilution that reduced infectivity by 50% using non-linear regression fitting in GraphPad Prism 7.0. Samples that failed to give 50% plaque reduction at 1:50 dilution were assigned as below the cutoff.

### ***IgM depletion assays***

To assess whether the NT activity was associated with IgM, plasma samples from “IgM alone with NT activity” patients were depleted of their IgM activity prior to NT assays as described previously (35). Briefly, heat-inactivated plasma was treated with 0.1M dithiothreitol (DTT) (Life Technologies Cat # R0861) to a final concentration of 2.5 mM to inactivate IgM, followed by confirmation of loss of IgM functionality. This plasma was then assessed for its NT activity as described above.

### ***Virological assays***

For viral recovery assays, 50ul of patient plasma was diluted with 450ul of DMEM (Invitrogen Cat # 11965-092) and inoculated onto the C6/36 (ATCC, Cat # CRL-1660) cells in 6 well tissue culture plates. Viral RNA was extracted from culture supernatant using viral RNA extraction kit (Qiagen Cat no-52906) and reverse transcribed into cDNA using revert Aid first strand cDNA synthesis kit(Thermo fisher Cat no-K1632). PCR was performed by semi-quantitate method using Chikv E1 specific primers (CHIK/E1-S 5'-

TACCCATTCATGTGGGGC-3'; CHIK/E1-C 5'-GCCTTTGTACACCACGATT-3) and the products were analyzed on a 2% agarose gel. Viral copy numbers were analyzed by real time PCR as reported earlier(36). For plasma PCR, RNA was extracted from sera using QIAamp Viral RNA Mini Kit as per manufacturer's instructions. PCR was performed as described previously (36, 37). Briefly, three sets of primers targeting E1 gene (930bp, 294bp, and 200bp) were used to confirm CHIKV positivity.

### ***Statistical Analysis***

Statistical analyses were performed using Graph Pad prism 7.0. Multiple groups were compared using analysis of variance (ANOVA) followed by Dunn's post-test. Correlation between two groups was calculated by Spearman correlation coefficient  $r$ . The unpaired analysis was done using Mann-Whitney test. Paired groups were compared by two tailed t test. Significance between proportions was calculated using Fisher's test. A  $p \leq 0.05$  was considered significant.

### ***Informed consent***

The study was approved from the institutional ethical committees (KIMS, HIMSAR, ICGEB) and the participants gave written informed consent.

### ***Author contributions***

KN - experiments and manuscript preparation; AC, PR, KMK - study design, interpretations and manuscript preparation; VJ and VHR - patient recruitment and follow-up; MK, NK, KG, RCR, KD, RS, SG, MI, AV, DM, CA, YC, ESR, HP, PS, PB, PS, SRB, AKP - data acquisition and analysis.

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Figures and Figure Legends: .

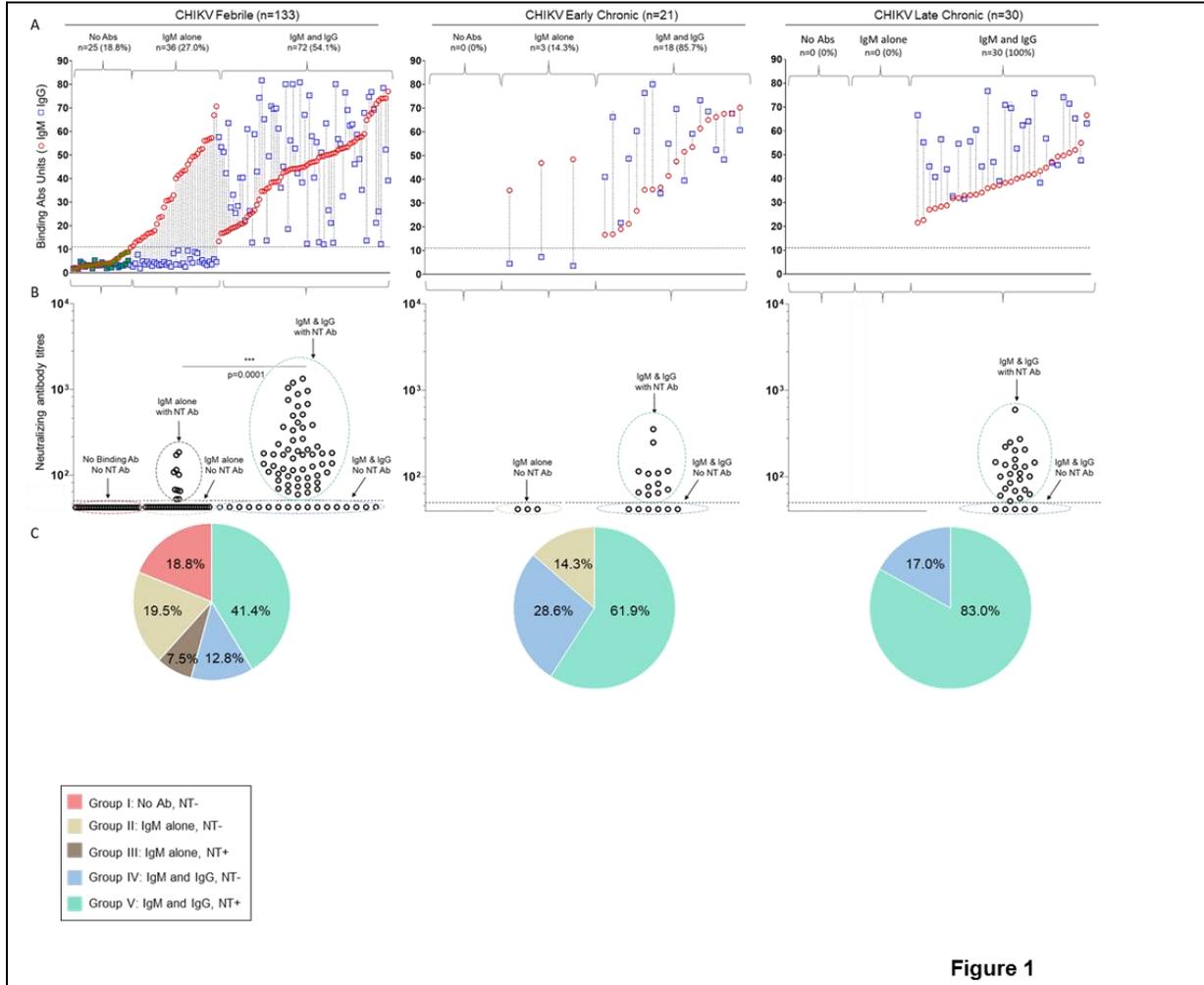


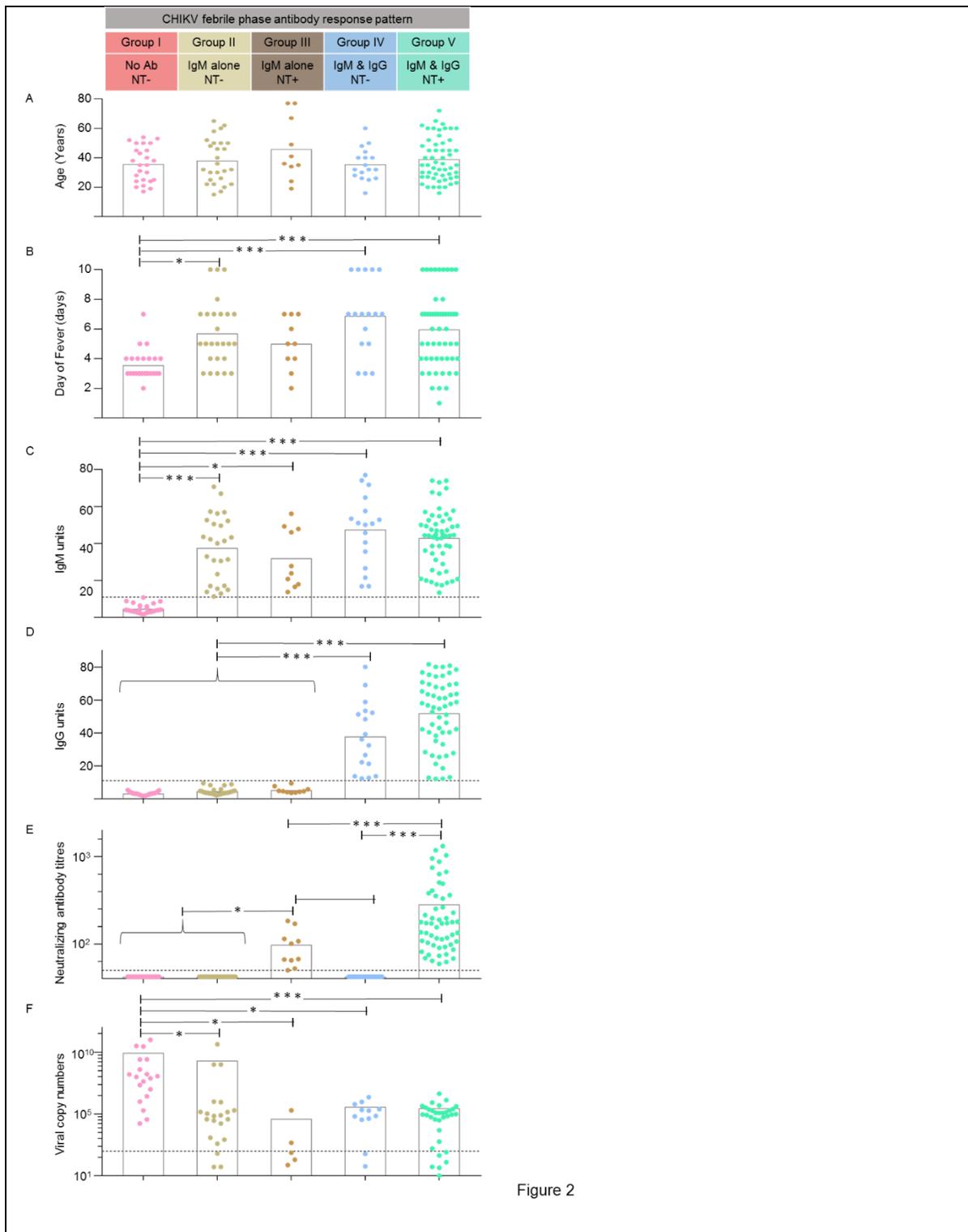
Figure 1

**Figure 1. Diversity of the antibody response patterns in chikungunya acute febrile versus chronic patients.**

**Panel A.** Paired analysis of CHIKV specific plasma IgM (red circles) and IgG (blue squares) values in individual CHIKV confirmed patients within the no Abs, IgM alone or IgM and IgG Ab response patterns in the acute febrile (left, n=133), early chronic (middle, n=21) and late chronic (right, n=30) phases. Within each antibody response pattern groups, the patients are stratified based on increasing order of IgM values on X-axis. Horizontal dotted line indicate assay cut-off for IgM and IgG. The samples that were also positive for CHIKV-PCR are indicated by green filled symbols.

**Panel B.** Evaluation of plasma CHIKV NT antibody activity in each of the patient groups that are described in panel A. Dotted gates were placed to further subgroup the patients based on a combination of IgM, IgG and NT activity. NT assay limit of detection is indicated by the horizontal dotted line. The NT Ab titers were significantly different between IgM alone group and IgM & IgG group in the acute febrile patients. Statistical significance was calculated by unpaired Mann-Whitney test.

**Panel C.** Relative proportion of the patients with each of the indicated antibody response pattern shown in Panel B among the CHIKV confirmed patients in febrile phase (Left panel), early chronic phase (Middle Panel) and late chronic phases (Right Panel).



**Figure 2. Characterization of age, days of fever (DOF), antibody response (IgM, IgG & NT) and Viral copy numbers in acute febrile phase.**

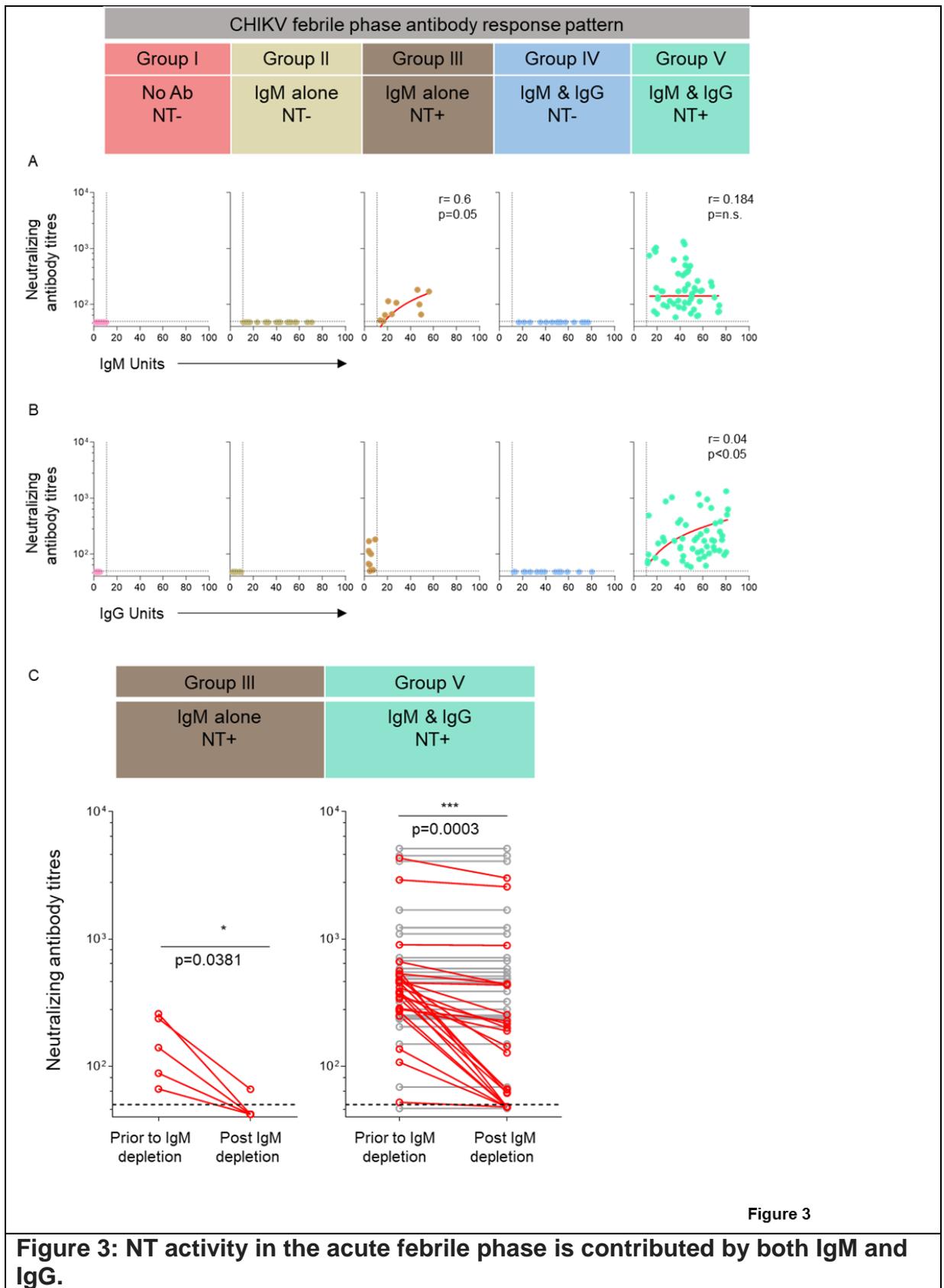
**Panel A to D :** Patients are divided into five indicated groups based on their acute febrile antibody response patterns. Group I (n= 25), Group II (n= 26), Group III (n=

10), Group IV (n= 17), Group V (n= 55). **Panel A**, Distribution of age. **Panel B**, Distribution of the Days of Fever. **Panel C**, IgM levels. **Panel D**, IgG levels. **Panel D**, PRNT<sub>50</sub> values

**Panel E**, Viral copy numbers where virus was recovered. Group -I (n=19), Group II (n=21), Group III (n=5) Group IV (n=12) and Group V (n=33 )

Mean values for each group are indicated by bars. Comparison between the groups was performed by Kruskal-Wallis one-way analysis of variance (ANOVA) with Dunn's post-test. \*\*\* indicate  $p < 0.0001$  and \* indicate  $p < 0.05$ . Each dot represents individual patient. Mean values are indicated by bars. Dotted lines indicate the assay cut-off limits for IgM, IgG positivity or for NT titers or for viral copy numbers.

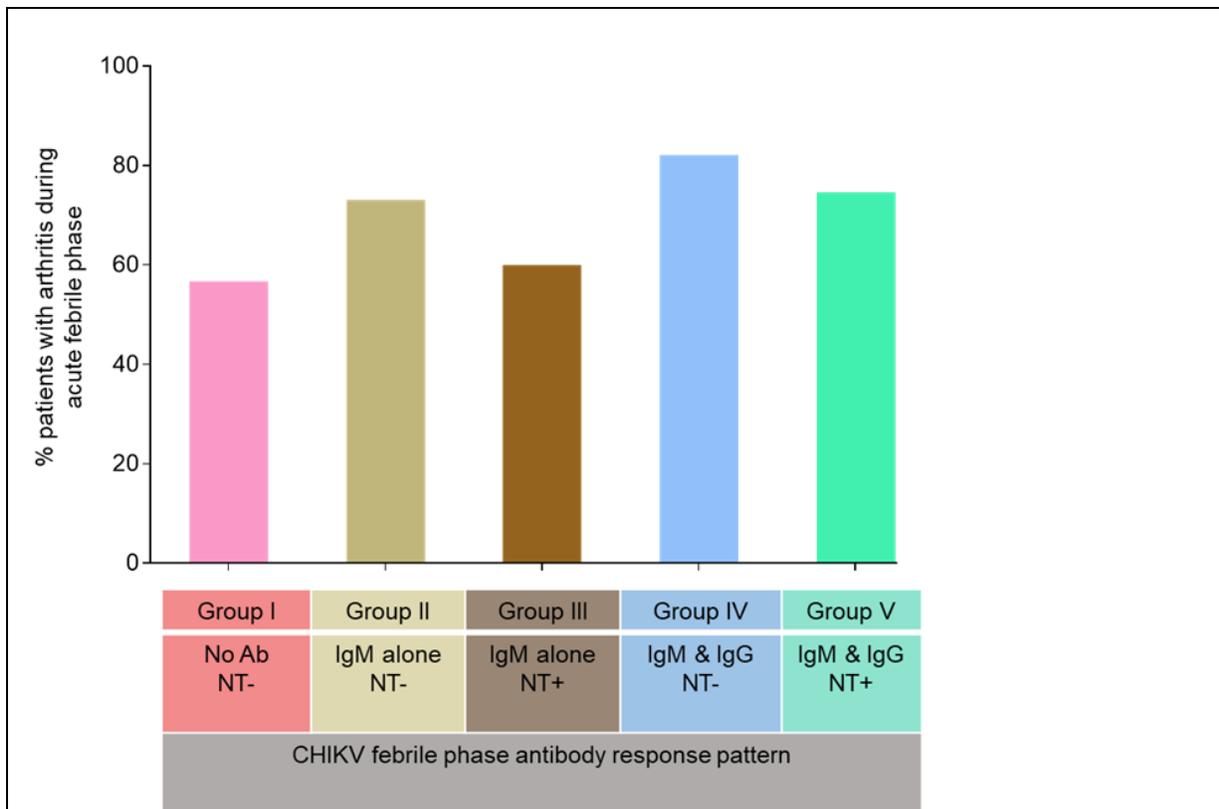
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**Panel A.** Analysis of correlation between NT Ab titers and IgM levels in plasma of patients from group I (n=25), group II (n=26), group III (n=10), group IV (n=17) and group V (n=55). Dotted line indicate assay cut-off. Spearman correlation coefficient r was calculated. p values are indicated where significant.

**Panel B.** Analysis of correlation between NT Ab titers and IgG levels in plasma of patients from group I (n=25), group II (n=26), group III (n=10), group IV (n=17) and group V (n=55). Dotted line indicates assay cut-off. Spearman correlation coefficient r was calculated, and p values are indicated where significant.

**Panel C.** NT Ab titers prior to and post IgM depletion. Plasma of group III patients (left, n= 5) and group V patients (right, n=50) were evaluated for NT activity prior to and post IgM destruction. Dotted line indicates assay cut-off. The patients in who NT titers decreased after IgM depletion are marked in red (100% in group III & 48% in group V). Paired two tailed t test was performed to evaluate statistical significance.



**Figure 4**

**Figure 4. Acute febrile arthritis characteristics of the patients with individual antibody response patterns.**

*Proportion of the arthritis cases in each of the indicated groups during the febrile phase. IgM & IgG NT<sup>-</sup> (n=17); IgM & IgG NT<sup>+</sup> (n= 55); IgM only NT<sup>-</sup> (n=26); IgM only NT<sup>+</sup>(n=10); and No Abs (n=25). Statistical significance was calculated by chi square test. The arthritis case frequency among the groups was not statistically significant between any of the groups (p= > 0.05).*

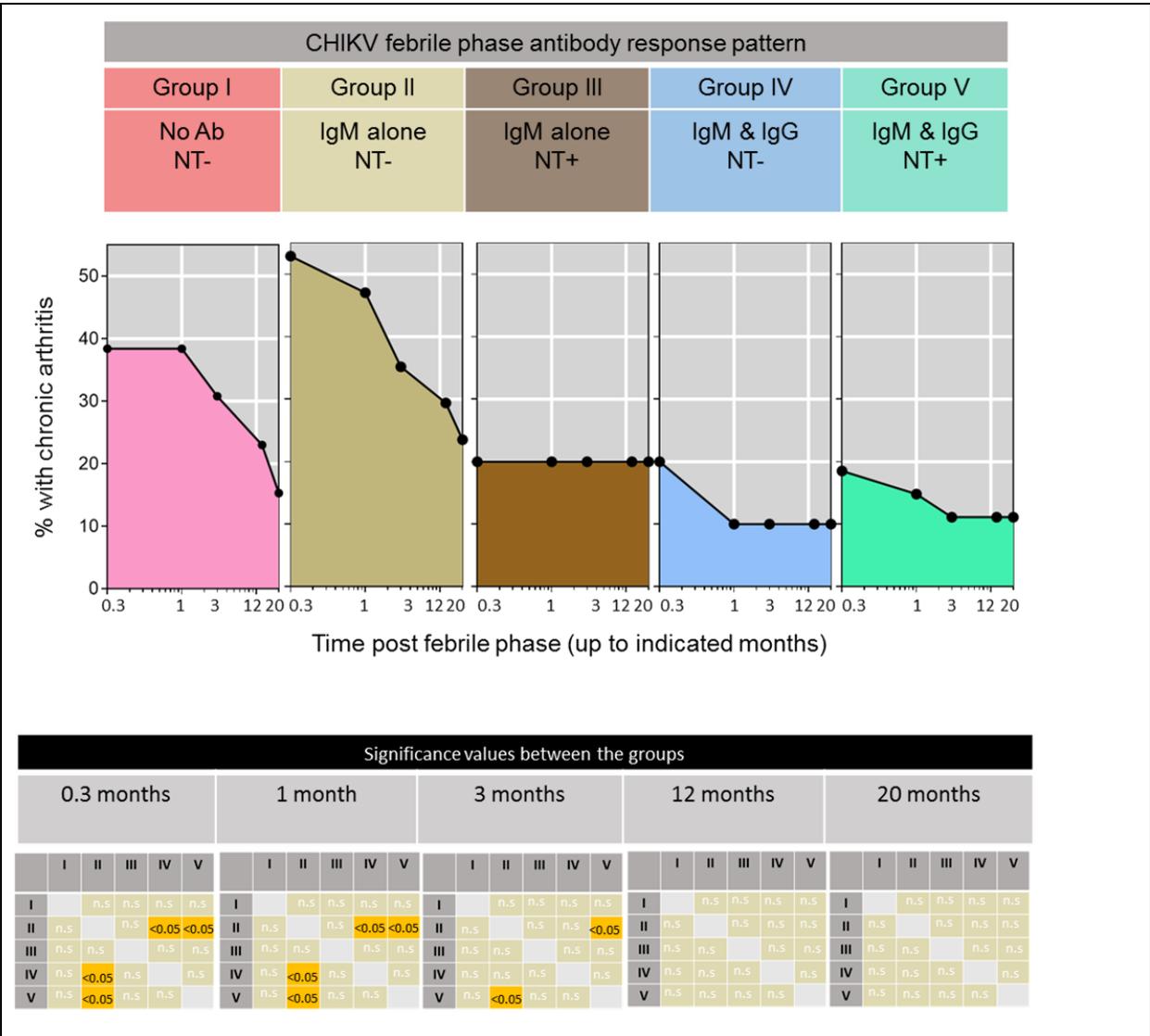


Figure 5

**Figure 5. Association of initial febrile antibody response patterns with the downstream fate of protection against developing chronic arthritis.**

Patients that were followed for more than 20 months as described in methods are subdivided into the indicated five groups based on their initial febrile antibody response pattern. Group I (n= 13), Group II (n= 17), Group III (n= 5), Group IV (n= 10), Group V (n= 27). The proportion of the patients in each of the group with chronic arthritis is indicated at indicated times post resolution of febrile fever. Significant values for the proportion of arthritis cases between different groups at 0.3-month, 1 month, 3 months, 12 months, and 20 months are indicated in checker boards below the figure.

Significance was calculated using Fisher's exact test of independence. p values < 0.05 are indicated by yellow color. Where p values were non-significant, they were indicated by gray shade.