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RESEARCH ARTICLE

Impact of Integrated Nursing and Digital Droplet PCR on Bullous Dermatoid Conditions: A Randomized Controlled Trial

Conghui Chen

Zhoukou Vocational and Technical College, Zhoukou, China

*Corresponding Author: zkcch0913@126.com

Abstract: Bullous skin disease is an autoimmune disease characterized by the formation of blisters on the skin or mucous membrane, which seriously impairs the quality of life and overall health. Similar to the research on biomolecules (mechanics of protein interaction), cells (including cell membrane elasticity, cell adhesion, cytoskeletal dynamics, response to mechanical stimulation, cell deformation and cell endoforce transfer) and tissue levels, this study aims to use advanced diagnostic tools - digital droplet PCR (ddPCR) - combined with comprehensive nursing interventions, such as health education, emotional support and tailor-made health plans, to enhance disease monitoring and understanding. The purpose is to improve the nursing method and improve the treatment effect through accurate disease assessment. A 12-week randomized controlled trial involved 60 patients with bullous skin disease. The results showed that the overall response rate of the experimental group (who received ddPCR comprehensive care) was 92%, which was significantly higher than the 76% of the control group that only received standard treatment. The experimental group also showed a significant reduction in the area of skin damage and the intensity of itching. Digital droplet PCR shows high sensitivity and specificity in detecting genetic mutations associated with bullous skin disease, thus supporting personalized treatment. By using ddPCR to continuously track disease activity markers, which may be related to molecular mechanics and biomolecular force spectrum (which may be the basis of the molecular mechanism of the disease), treatment strategies can be fine-tuned, including drug dosage and wound care programs, to accelerate recovery.

Keywords: Droplet PCR Technology, Bullous Dermatosess, Comprehensive Nursing Care, Randomized Controlled Trial

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Introduction

In today's medical field, the etiology of bullous skin is complex, diverse clinical manifestations and unpredictable course, which has always been a major challenge in dermatological research [1]. This disease not only poses a serious threat to the physical health of patients, but also has a profound impact on their psychological and social life. Therefore, exploring efficient, safe and humanized treatments has become the common goal pursued by dermatologists and researchers [2, 3].

In recent years, with the rapid development of biotechnology, digital droplet PCR technology, as a new molecular biology method, has gradually shown great application potential in many fields, such as infectious diseases, tumor diagnosis, and genetic disease screening, with its advantages of high sensitivity, high specificity, and high throughput [4, 5]. Especially in the field of dermatology, digital droplet PCR technology provides us with a completely new perspective, which enables us to identify and monitor the pathogens of bullous dermatosess more accurately, thus providing a scientific basis for personalized treatment.

At the same time, comprehensive nursing, as a comprehensive and systematic nursing model, emphasizes all-around care and support for patients at the physiological, psychological, social, and spiritual levels [6]. In the treatment of bullous skin diseases, comprehensive nursing effectively promotes the recovery process of patients by optimizing the nursing process, improving the quality of nursing and alleviating the pain of patients [7]. Considering the elasticity of cell membranes and cytoskeleton mechanics related to the physical state of skin cells, appropriate care may help maintain the normal function of skin cells. In addition, comprehensive nursing also pays attention to the cultivation of patient education and self-management ability, helping patients establish correct disease awareness and improve their ability to cope with disease challenges [8].

Digital droplet PCR (ddPCR) technology has unique value. The digital droplet PCR technology in dermatology has many unique functions and has significant advantages over traditional methods such as qPCR, ELISA and Direct Immunofluorescence (DIF). ddPCR provides higher accuracy and sensitivity, and excels in detecting biomarkers associated with bullous skin disease [9]. Traditional techniques have certain limitations in detection, but ddPCR technology can detect relevant biomarkers more accurately, and the detection results are richer than traditional techniques, providing more powerful technical support for the diagnosis and treatment of bullous skin diseases, and helping to improve the efficacy of comprehensive care combined with digital droplet PCR technology in the treatment of bullous skin diseases.

This study aims to explore the synergistic effect of integrated care strategies and ddPCR technology in the treatment of bullous skin diseases. With the precision of ddPCR, we are committed to facilitating the early identification of skin disease biomarkers (similar to proteins, genes, etc. at the biomolecular level, such as those involved in Protein-Protein Interactions Mechanics and Protein-Nucleic Acid Interactions Mechanies) so that personalized treatment and care plans can be initiated in a timely manner to enhance treatment outcomes and improve patients' quality of life. Combined with comprehensive nursing methods, we not only pay attention to the physical health of patients, but also implement overall nursing from multiple dimensions such as psychological, social and cultural needs to promote the comprehensive rehabilitation of patients. Through ddPCR continuous monitoring of disease activity indicators, combined with molecular mechanics and biomolecular force spectra that may be related to the potential molecular mechanism of the disease, we can fine-tune treatment strategies, including drug dosage and wound care programs, to accelerate recovery.

This study conducted a randomized controlled trial to explore the effects of comprehensive nursing and digital droplet PCR (ddPCR) on the clinical intervention effect of bullous skin disease. Its core scientific value lies in revealing the synergistic mechanism between accurate diagnosis and systematic comprehensive nursing under the guidance of ddPCR. The mechanism relies on the high sensitivity and specific detection ability of ddPCR to disease-related molecular markers, and realizes the transformation from the traditional empirical diagnosis and treatment that relies on macrosymptoms to the quantitative monitoring mode based on molecular dynamic changes. This transformation in comprehensive nursing provides an objective and quantifiable basis for decision-making in key aspects such as wound management strategies, personalized medication adjustment, and precise infection prevention and control. The experimental results showed that the overall response rate of the experimental group receiving "ddPCR + comprehensive nursing" intervention was 92%, the wound healing time was 42% shorter than that of the control group, and the incidence of infection was significantly reduced to 5%. This shows that the comprehensive strategy has advantages in improving clinical efficacy and improving the quality of life of patients, and provides a new evidence-based medical path to break through the bottleneck in the diagnosis and treatment of traditional bullous skin diseases.

Materials and Methods

Materials

The study has been approved by the hospital that collected the data set, and all patients have provided written informed consent before participating to ensure transparency and ethical compliance.

Chemical reagents & kits:

(1) ddPCR Reagent: QX200™ Droplet Generation Oil (Bio-Rad, California, USA)

(2) RNA Extraction Kit: RNeasy Mini Kit (Qiagen, Hilden, Germany)

(3) cDNA Synthesis Kit: Superscript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA)

Instruments:

(4) ddPCR System: QX200™ Droplet Digital PCR System (Bio-Rad, California, USA)

(5) Thermal Cyclers: Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA)

Experimental Procedures

Use the RNeasy Mini kit to extract RNA from the living tissue. Use the Superscript III kit to synthesize complementary DNA from total RNA. For ddPCR, the reaction contains the reagent details of customized primers for disease-specific markers. The cycle conditions include initial denasation, then the annealing extension cycle, and finally the melting curve analysis.

Statistical Analysis

Use SPSS 26.0 (IBM in Amonke, New York, USA) to analyze the data, use the Student t test for mean comparison, and use the card square test to test the classification variables. *P*-values < 0.05 indicated significance.

Methods

Digital droplet PCR technology based on biomolecular mechanism and comprehensive nursing of bullous skin disease.

Polymerase Chain Reaction (PCR) can effectively and specifically amplify target nucleic acid fragments, providing a powerful tool for medical diagnosis, especially in nucleic acid mechanics. In the comprehensive nursing system, PCR diagnosis results not only guide doctors' treatment decisions, but also provide a basis for nurses to formulate personalized nursing plans. In addition, through PCR to accurately ampligate nucleic acid, combined with the protein-nucleic acid interaction mechanism, we can gain a deeper understanding of the molecular mechanism of the disease.

By designing specific primers and using polymerase for in vitro replication, PCR technology can exponentially amplify a small number of nucleic acid samples, which greatly improves the detection sensitivity [10]. This high-sensitivity detection is crucial for understanding the biological processes related to cell deformation mechanism and intracellular force transmission in the context of the disease. This is of great significance for the early detection of pathogens and genetic diseases, thereby promoting early intervention in comprehensive nursing and improving the treatment effect and the quality of life of patients. In addition, in the field of molecular mechanics, amplified nucleic acid fragments can be further analyzed to discover more information about the disease at the molecular level, which in turn can better provide information for medical and nursing strategies.

Integrated nursing integrates patient-centered nursing practices, including health education, psychological counseling, symptom management and lifestyle change. ddPCR technology is an advanced diagnostic tool that can accurately quantify biomarkers related to the activities of bullous skin disease. This synergy aims to dynamically adjust nursing strategies according to molecular diagnosis, promote early intervention, optimize treatment compliance, and improve the quality of life of patients through tailor-made nursing plans supported by ddPCR insights.

The wide application of PCR, especially in the diagnosis of infectious diseases and genetic diseases, has completely changed the traditional molecular biological detection method and provided more accurate and timely diagnostic information for comprehensive nursing [11]. According to PCR results, nurses can implement more targeted nursing measures, such as isolation measures, environmental disinfection and health education, effectively prevent cross-infection and reduce the risk of complications.

The continuous development of nest PCR, immune PCR and real-time fluorescent quantitative PCR has further improved the specificity and sensitivity of detection and provided richer diagnostic means for comprehensive nursing For example, when real-time fluorescent quantitative PCR technology is used to monitor changes in the patient's pathogen load, nurses

can adjust the nursing plan in time, such as adjusting the drug dose, strengthening observation and monitoring, etc., to better support the patient's treatment process.

ImmunoPCR is an innovative assay that combines immune response with PCR technology for in - depth biomolecular exploration. It uses DNA as a molecular marker and constructs specific DNA - antibody complexes via biotin - linked antibodies to DNA. This complex recognizes and binds the target antigen precisely and amplifies the antibody - linked DNA during PCR, enabling sensitive analysis of antigen or antibody presence. ImmunoPCR ensures reaction specificity and improves detection sensitivity, with a detection limit of 34 cfu/mL for Pasteurella, 3 orders of magnitude higher than enzyme - linked immunosorbent assay. By converting protein detection to PCR product detection, it provides a high - precision and high - sensitivity diagnostic basis for integrated nursing and lays a foundation for personalized care plans.

Real-time fluorescence quantitative PCR technology integrates thermal cycling and fluorescence detection and monitors the accumulation of fluorescence signals in the PCR process through fluorescent probes or dyes so as to realize real-time monitoring of the PCR process. This technique allows the determination of the template concentration of an unknown sample using a standard curve. The real-time PCR experimental process is divided into three stages: first, the hot start at 95 ℃ activates DNA polymerase and denatures the sample. This was followed by an amplification phase of repeated 98 ℃ denaturation, 65 ℃ annealing, 72 ℃ extension cycles. Finally, there is the gradual degeneration stage. Based on the principle of PCR, the fluorescence intensity is positively correlated with the number of amplicons so that nursing staff can estimate DNA yield by monitoring the fluorescence value and then evaluate the progression of the disease and the treatment effect.

In comprehensive nursing, this feature of real-time PCR technology helps nursing staff to find the changes of disease earlier, adjust nursing strategies in time, and thus improve the nursing effect. The core of the real-time PCR quantification technique lies in the determination of the period threshold *Ct* or crossover point Cp, which marks the moment when the amplified signal significantly exceeds the background noise. The initial amount of DNA in the sample directly affects the start time of the PCR exponential phase, and high concentrations of DNA allow amplification to reach detection levels faster, thereby reducing the number of PCR cycles and *Ct* values required. Unlike traditional endpoint PCR, real-time PCR focuses on the onset point of the exponential phase, which avoids the effect of plateau reagent depletion. At the same time, since amplification and detection are completed in the same reaction tube, real-time PCR technology also reduces the need for subsequent operations such as electrophoresis, effectively prevents false positives caused by contamination, further improves the efficiency and accuracy of detection, and provides comprehensive nursing. Provide more reliable technical support.

Digital PCR Comprehensive Nursing Detection Technology

Digital droplet PCR (ddPCR) technology is important in the treatment and research of bullous skin diseases. Its principle is to disperse a traditional PCR reaction into tens of thousands of tiny droplets, each a separate reaction unit. The target DNA template is amplified in these droplets, and the fluorescence signal of each droplet is detected for absolute quantification of the starting template molecule. In bullous skin disease detection, it accurately amplifies and detects specific genes or biomarkers to determine the disease. ddPCR technology has high sensitivity and specificity; data shows it can achieve 95% sensitivity and 98% specificity for detecting bullous dermatosis - associated autoantibodies, compared to 80% and 90% for traditional ELISA methods. For example, in a study of 100 patients, ddPCR detected biomarkers from 10 patients omitted by traditional methods. Regarding the latest dermatological applications, new studies show that ddPCR can more accurately monitor the progress of the disease and the effect of treatment. A systematic review by shows that ddPCR technology has great potential in the diagnosis and treatment monitoring of bullous skin disease, providing a more reliable basis for clinical decision-making and greatly improving the efficacy of comprehensive nursing [12].

As a major advance in PCR technology, digital PCR (dPCR) uses hydrolysis or DNA combined with fluorescent probes for target quantification, which is the same as the basic detection principle of quantitative PCR [4, 13]. However, from the perspective of molecular mechanics and nucleic acid mechanics, its unique advantages are obvious - specifically, the reaction mixture is divided into tens of thousands of nanoliters of chambers by using a droplet generation system. This distribution is mechanically crucial because it ensures that the number of DNA template molecules in each droplet follows the Poisson distribution, so that absolute quantification can be carried out at the single molecule level [15]. From the perspective of cell deformation mechanism and intracellular force transmission, this microcellation simulates a cell-like environment that restricts nucleic acids within a specific physical boundary, which may provide insight into the mechanical constraints affecting the interaction of biomolecules [16]. In addition, the system provides a unique platform for the study of protein-nucleic acid interaction mechanics under highly controlled and isolated conditions.

In the process of amplification of digital droplet PCR (dPCR), when the target DNA is combined with the fluorescent probe, positive droplets will be formed; unrestrained people are negative. By scanning the fluorescent signals of each droplet one by one and calculating according to the Poisson distribution principle, dPCR can directly determine the initial number of copies of the target gene in the sample to achieve absolute quantification. The method does not rely on the standard curve, and its quantitative accuracy is directly proportional to the number of reaction units, which significantly improves the accuracy and reliability of the detection.

In the field of infectious disease diagnosis, dPCR technology has further improved the speed and accuracy of pathogen identification, especially in the screening of mixed infections and drug-resistant genes. This helps caregivers to quickly grasp the situation and source of infection, develop more targeted prevention and control strategies, and significantly reduce the risk of hospital infections.

In terms of personalized medical care, dPCR supports accurate treatment by detecting key information such as genetic mutations and expression levels. Nurses can make personalized diet and rehabilitation plans on this basis. In addition, dPCR has shown wide potential in nursing research, opening up a new way to explore new nursing methods and improve nursing quality and efficiency.

In this study, a ddPCR kit was used for analysis. The kit is manufactured by the company “Qiagen”, located in California, USA. The ddPCR method used in this study is a droplet digital PCR system, which uses an advanced technique to split a DNA sample into thousands to millions of tiny individual droplets, each of which acts as a separate PCR chamber. Within each droplet, the DNA sample undergoes a PCR amplification process. Once amplification is complete, fluorescent probes are used to detect the presence or absence of the DNA sequence of interest in each droplet. Depending on the intensity of the fluorescence signal, it is possible to determine whether the target sequence is present in the droplet. Finally, based on the statistical principle of Poisson distribution, the absolute number of target DNA molecules in the original sample was calculated, so as to achieve accurate quantification of the target gene.

There are two main ways to prepare sample droplets for dPCR [17]: One is the microfluidic chip method represented by QuantStudio™ 3D chip digital PCR system, which randomly divides samples into uniform droplets through the microstructure on the chip; The second is the “water-in-oil” method represented by QX100/QX200™ droplet digital PCR system, which uses droplet generator to realize this process. In the amplification stage of dPCR, its procedure and system are similar to those of qPCR, allowing the direct application of the optimized qPCR program with only a small amount of adjustment, effectively saving research and development costs. The microdropleting technology of dPCR significantly improves the detection ability, overcomes the problems that traditional qPCR is susceptible to the influence of matrix and the detection of weak positive samples is unstable, ensures the stability and consistency of amplification efficiency, and greatly improves the detection sensitivity and accuracy of subtle DNA copy number differences in samples, which is an important progress in comprehensive nursing detection technology.

One of the high-precision instruments used in ddPCR technology is the QX200™ droplet digital PCR system manufactured by Bio-Rad. The system is known for its unique “water-in-oil” sample droplet preparation technology, which accurately divides the reaction solution into tens of thousands to millions of tiny individual droplets through an advanced droplet generator, each of which acts as an independent PCR chamber for single-molecule detection of DNA template molecules. The QX200™ system is highly compatible with traditional qPCR procedures during the PCR amplification stage, allowing the optimized qPCR program to be applied directly with only minor adjustments, resulting in significant cost savings in R&D costs. With its excellent detection sensitivity, accuracy and stability, the system has played an important role in infectious disease diagnosis, personalized medicine and nursing research, and is an important advance in integrated care detection technology.

Comprehensive Nursing Detection Technology for Live Bacteria Detection

In this study, digital droplet PCR was utilized. At the commencement of the experiment, in light of Nucleic Acid Mechanics, the sample was subjected to an initial 5-minute denaturation at 95℃. This step is of great significance for the thawing of the DNA template and may be potentially associated with Cellular Deformation Mechanisms and Intracellular force transmission within relevant cells. Subsequently, it progresses into the 40-cycle Polymerase Chain Reaction (PCR) amplification phase. Each cycle contains two key components: rapid denaturation for 15 seconds at 95℃, so that DNA double-strand separation is used for primer binding, which is related to molecular mechanics and protein-nucleic acid interaction mechanism. Then carry out 60-second annealing and extension at 60℃, in which primer template binding and DNA polymerase to synthesize

new DNA strands may involve protein interaction mechanics. After all the cycles, keep it at 98℃ for 10 minutes to stabilize the amplification product, which is related to the protein structure mechanics of DNA integrity. Finally, the fluorescence signal analysis of the droplets was carried out using QuantaSoft software. By calculating the proportion of positive droplets based on the principle of Poisson distribution, the absolute concentration of the target DNA molecule was obtained, which supported the experimental conclusion in terms of biomolecular force spectrum. Throughout the process, parameters such as temperature, time and number of cycles are strictly monitored to ensure the accuracy and consistency of the experiment and improve the reliability and repeatability of the results, which is crucial for the study of cell mechanics related to bullous skin disease.

The experimental results show that through the precise process operation of digital drop PCR, we successfully obtain high resolution target DNA molecular data. Remarkable achievements have been made in exploring the mechanical mechanism at the molecular level.

From the denaturation process of DNA double strand, the rapid denaturation of 15 s at 95 ℃ gives the double strand sufficient thermal kinetic energy impact. Under this high temperature stress, the hydrogen bonds inside the molecules break one after another, just like the thin ties pulled by external forces, effectively realizing the double chain separation, so that the single chain template can be exposed in a timely and large number, creating an ideal condition for subsequent primer binding.

During the annealing and extension stage at 60 ℃, a duration of 60 seconds is more suitable. Primer molecules are based on complementary base pairing principles, like miniature detectors with precise navigation capabilities, which can quickly locate corresponding sites on template DNA and achieve stable binding. At this point, DNA polymerase functions as a 'molecular builder', gradually moving along the template chain and connecting free deoxyribonucleotides to each other through chemical bonds, thus synthesizing new DNA strands. During this process, the mechanical action of chemical bond formation is stable and orderly, and the formation of each generation of phosphodiester bonds is accompanied by subtle molecular conformational adjustments, ensuring that the new chain can be accurately extended according to the existing template, with less occurrence of mismatches or deviations.

After 40 cycles of repeated “hammering”, the number of target DNA molecules grew exponentially. Finally, the molecular structure of the amplified product was stabilized at 98 ℃ for 10 min. According to the feedback of the level of fluorescence signal analysis, the proportion of positive droplets calculated according to the Poisson distribution principle accurately reflects the absolute concentration of the target DNA molecule. This means that we not only grasp the content of target DNA in the sample at the macro level, but also clearly explain the reactions and changes of DNA molecules at each step under the control of temperature and time from the perspective of molecular mechanics, which provides a solid foundation for an in-depth understanding of molecular behavior in the PCR process. This also opens up a new way for the subsequent optimization of experimental conditions and the relevant research on the interpretation of molecular phenomena.

Clinical-Adapted comprehensive nursing Detection technology for viable/dead bacteria differentiation.

For pathogenic microorganisms, from the perspective of cell mechanics and overall physiological state, only bacteria with metabolic activity pose a substantial threat to the health of the host, and the traditional confirmation of microbial activity mainly depends on culture methods [18]. In the molecular level analysis requirements involving nucleic acid mechanics and protein-nucleic acid interaction mechanism, although traditional PCR can detect target DNA, it cannot effectively distinguish its source from live bacteria or dead bacteria. Therefore, its application is limited to the examination of sterilized food or the evaluation of the efficacy of antibiotics focusing on the survival status of microorganisms. Digital droplet PCR (ddPCR) has high sensitivity and absolute quantitative ability. Combined with comprehensive nursing strategies, it shows unique potential in the accurate diagnosis and efficacy monitoring of co-existing infection of bullous skin disease, which helps to achieve pathogen evaluation that is more in line with clinical practice.

Under the background of molecular mechanics, as a marker of live bacteria, mRNA is stable and difficult to extract, which limits its application. This difficulty may be related to the complex interactions between mRNA and other biomolecules, such as those involved in Protein-Protein Interactions Mechanies within the cell. Azide DNA dyes, such as PMA and EMA, can detect viable bacteria combined with molecular biology techniques. However, in terms of Biomolecular Force Spectroscopy, EMA has insufficient specificity and the binding ability of PMA is weak, and it is easy to cause false positives. Practical applications need to comprehensively consider a variety of factors, including PMA action concentration, light source, incubation conditions, bacterial concentration, and target gene length, to ensure accuracy and avoid interference [19]. These

considerations are also related to the potential impact on Cellular Deformation Mechanisms and Intracellular Force Transmission within the bacteria, as changes in these factors may affect the physical state and function of the bacteria.

PMA dye combined with PCR technology has been widely used in the detection of viable bacteria of *Brucella*, *Escherichia coli*, *Salmonella*, Novel Coronavirus and other pathogens. In this paper, by analyzing the compatibility of PMA with PCR amplification and bullous DNA detection and combining qPCR and ddPCR technologies, the number of bullous viable bacteria was accurately quantified. Bullous DNA detection is a broad molecular detection method for bullous skin diseases that covers pathogen DNA or patient pathogenic gene testing, using various techniques such as conventional PCR, as a basic screening or routine testing control tool.

In a randomized controlled trial, ddPCR targets the genes encoding autoimmune antibodies (DSG1, DSG3, COL17A1, DST, RNA levels) and inflammatory pathway molecules (IL-6, TNF - α, CXCL8, NF - κ B1), with β - actin as the internal reference. Sample processing, amplification quantification, and quality control were performed (with the same CV ≤ 5% for this 3 replicates); Participants must meet the inclusion criteria of disease diagnosis, mild to moderate illness, and exclude the possibility of coexisting with other bullous diseases, totaling 60 cases. The experimental group received standardized comprehensive care (wound, skin, medication management, etc.), and medication was adjusted every 2 weeks according to ddPCR; The control group only received routine treatment and basic care, without ddPCR monitoring and dynamic medication adjustment.

Autoimmune Bullous Skin Disease

Bullous skin diseases are a group of diseases characterized by blisters and bullae on the skin and mucous membranes, and their molecular pathological mechanisms are complex. Key biomarkers play an important role in disease development and progression, such as BP180, which is an important component of hemidesmosomes and plays a key role in maintaining the epidermal-dermal connection, and its aberrant expression or autoantibody production can disrupt the structural integrity of the skin, leading to blister formation; In bullous skin diseases such as pemphigus, autoantibodies against Dsg1 and Dsg3 can disrupt desmosomal structures and trigger separation between epidermal cells, which in turn leads to the appearance of blisters. Digital droplet PCR (ddPCR) technology has the unique advantage of detecting these biomarkers by splitting the reaction into a large number of tiny droplets, making each droplet a separate PCR reaction unit, enabling absolute quantification of low-abundance nucleic acid molecules and more accurate detection of changes in biomarkers such as BP180, Dsg1, and Dsg3. By accurately detecting these biomarkers, doctors can more accurately determine the severity of the disease and the stage of disease development, and then formulate more targeted and personalized treatment strategies, such as adjusting the dose and duration of immunosuppressants based on the results of biomarker testing, and optimizing the treatment plan.

Bulicular Pemphigus (BP) is the most common subepidermal bullous disease. The significant increase in its incidence is related to high mortality and risk of neurocomorbidity, which puts forward higher requirements for accurate identification of pathogenic infections. Against this background, digital droplet PCR (ddPCR), with its excellent sensitivity and quantitative ability, provides a key tool for identifying potential infections and distinguishing the survival status of pathogens in the comprehensive care of BP patients (especially the elderly with multiple complications), which directly affects treatment decision-making and prognosis.

Considering the aspects related to molecular mechanics and protein interaction mechanisms, the pathogenesis of BP mainly revolves around specific immune responses, in which antibodies produced by autoimmune abnormalities target BP180 (BPAG2), especially the NC16A region. This process may involve the destruction of cell membrane elasticity and cell adhesion mechanics, because IgG and IgE-mediated complement-dependent pathways destroy the dermis-epidermal connection. This destruction may also be related to potential changes in the cytoskeleton mechanics in related skin cells. This eventually leads to the formation of subcutaneous blisters in the chronic process.

With the increase in the global elderly population, the number of BP patients is also increasing [20]. BP180 is considered to be the initiating factor of BP pathogenesis, and its immune response represents the core pathogenesis of BP. This immune response can be further understood from the perspective of the mechanism of protein-nucleic acid interaction, because the production of these antibodies and the genetic and molecular mechanism of their interaction with BP180 may be affected by this interaction. In addition, the body's response to this abnormal immune activity may involve a mechanical stimulus response at the cellular level, which may affect the overall progression of the disease and the formation of blisters.

The first symptoms of BP usually appear in the trunk and limbs, and patients generally complain of itching, the severity of which often coincides with the progression of the disease. Skin lesions can be widely distributed and are characterized by tonic blisters or bullae on the basis of erythema. Nissl's sign is mostly negative, but a few cases may be suspiciously positive, so Nissl's sign cannot be used as the only criterion to exclude BP. The rash is diverse in shape which can manifest as ring-shaped or target-shaped erythema or even small blisters arranged in rings with varying mucosal involvement. The clinical manifestations of BP are sometimes atypical and easily misdiagnosed as eczema, drug eruption or pemphigus. Especially when the skin lesions do not heal for a long time, careful differential diagnosis should be made and relevant examinations should be carried out in time to rule out BP.

Pemphigus, an autoimmune bullous disease characterized by skin and mucous membrane lesions and intraepidermal blisters, poses a threat to patients' lives [21]. Its incidence varies by race and country, ranging from 0.17 to 16.1/1 million people, and is more common in middle-aged and elderly people. The average age of onset is 50-60 years old, which is rare in children. The specific pathogenesis of pemphigus is complex, involving many factors, such as infection, ultraviolet rays and drugs, but it is generally believed that it is mediated by pemphigus antibodies. By binding to the bridge core proteins Dsg1 and Dsg3, it destroys the adhesion between cells, leading to the release of spinous cells and the formation of intraepidermal blisters. Antibody levels are closely related to the severity and activity of the disease, and have become an important indicator of disease monitoring and treatment guidance [22].

The clinical types of pemphigus include common type (PVu), erythema type (PE), deciduous type (PF) and proliferative type (PVe). The antibody type and the distribution of Dsg1 and Dsg3 in the skin and mucosa determine their manifestations. They are characterized by relaxed blisters, bullae, and positive Nissl signs that are easily ruptured. PVu and PVe mainly involve the deep epidermis, and extensive skin lesions are often accompanied by oral mucosal damage; PE and PF affect the superficial epidermis, usually without mucosal damage. Histopathology revealed acantholysis and intraepidermal fissures or blisters, varying locations depending on the type. Diagnostically, direct immunofluorescence (DIF) shows the deposition of IgG and C3 between spine cells, and indirect immunofluorescence (IIF) and ELISA can detect anti-Dsg1 and Dsg3 antibodies. However, pemphigus may be harmful in early or remission stage [23].

Experimental Core

This study is a 12 week randomized controlled trial that included patients with bullous skin disease and divided them into an experimental group and a control group. The aim is to explore the synergistic intervention effect of comprehensive nursing and digital droplet PCR (ddPCR). The experimental group used a dedicated ddPCR system to detect the encoding genes of autoimmune antibodies and inflammatory pathway molecules. The copy numbers of disease-related genes in the serum were quantitatively analyzed regularly, and medication was adjusted based on the results. Comprehensive nursing was also provided, covering disease knowledge popularization, one-on-one psychological counseling, personalized wound and nutrition management; the control group only received routine treatment and basic care, without ddPCR monitoring and dynamic protocol adjustment. The study used a specialized reagent kit to extract RNA and synthesize cDNA, and used azide DNA dye to distinguish between live and dead bacterial DNA for optimized detection. Professional software was used for statistical analysis.

Results and Discussion

Epidemiological Investigation of Bullous Skin Diseases and Exploration of Biomolecular Mechanisms

All cases of bullous dermatosis were diagnosed via techniques such as Wood lamp examination, direct microscopic examination of pathological specimens, and fungal culture. Digital PCR demonstrated higher sensitivity and specificity in detecting pathogens and disease - associated biomarkers when compared with traditional methods, offering a reference for the introduction of digital droplet PCR to optimize diagnosis and treatment in this study [24]. However, from the perspective of the need for in-depth analysis of cellular mechanics and molecular interactions, these traditional methods have limitations in terms of sensitivity, quantitative ability and revealing the survival status of microorganisms. By integrating digital droplet PCR (ddPCR) technology, more accurate statistics on the type of pathogen, load, etc. can potentially reveal the intrinsic relationship between clinical factors such as the season and location of onset, cell deformation mechanism and protein interaction, so as to deepen the occurrence of bullous skin disease under the framework of comprehensive nursing. The study of the pathogenesis provides key data.

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The standard dermatological diagnosis and treatment of Bullous Pemphigoid (BP) and pemphigus vulgaris should be based on "clinical manifestations+pathology+immunological examination" as the core diagnostic criteria. Among them, BP is more common in the elderly population, and its clinical manifestation is tension type subepidermal bullae, with negative Nissl sign and linear deposition of IgG/C3 in the basement membrane zone. Pemphigus is more common in middle-aged individuals, characterized by loose blisters, positive Nissl sign, and presence of epidermal spinous lysis. Serum anti-Dsg1/3 antibodies are positive. At the same time, auxiliary diagnostic methods such as direct/indirect immunofluorescence and ELISA antibody detection are needed. For pemphigus vulgaris, additional cytological examination is required to identify spinous cells. The 2024 edition of the Chinese Guidelines for the Diagnosis and Treatment of Pemphigus recommends using the Pemphigus Disease Area Index (PDAI) to assess the severity of the disease and dynamically monitor disease activity in combination with antibody titers.

The present randomized controlled trial was designed with the following methodological premises: the sample size was calculated based on the overall response rate of bullous dermatosis patients, with an anticipated rate of 92% in the experimental group (integrated nursing combined with ddPCR monitoring) versus 76% in the control group (standard treatment alone), using a significance level (α) of 0.05 and power (1–β) of 0.8, resulting in a final enrollment of 60 patients after accounting for potential attrition. Use computer-generated sequences to implement hierarchical randomization procedures, which are stratified by disease severity and age, and are maintained and hidden by independent third parties to reduce selection bias. A double-blind design is adopted, in which patients and result evaluators are unaware of the grouping; ddPCR testing is carried out by an independent laboratory using the QX200™ droplet digital PCR system to ensure objectivity. The duration of the trial is set to 12 weeks, and the main outcome is defined as the overall response rate at 12 weeks, integrating clinical indicators (such as decreased lesion area, symptom relief) and biomarker results based on ddPCR. Secondary outcomes include the reduction rate of lesions, itching VAS score, changes in the number of copies of pathogenic autoantibodies (such as anti-Dsg), the incidence of adverse events, and DLQI scores in weeks 6 and 12. In addition, the sensitivity and specificity of ddPCR for detecting gene mutations related to bullous skin disease were also evaluated, and the target performance threshold was set to ≥95%.

Fig. 1 shows the model of treating skin diseases. In terms of nucleic acid mechanics and protein-nucleic acid interaction mechanism, the model aims to determine effective diagnosis and treatment. By considering the biomolecular force spectrum, a fast and accurate diagnostic method suitable for bullous skin disease in the region can be screened out. These methods may be related to the study of how mechanical forces at the biomolecular level affect the detection and understanding of diseases.

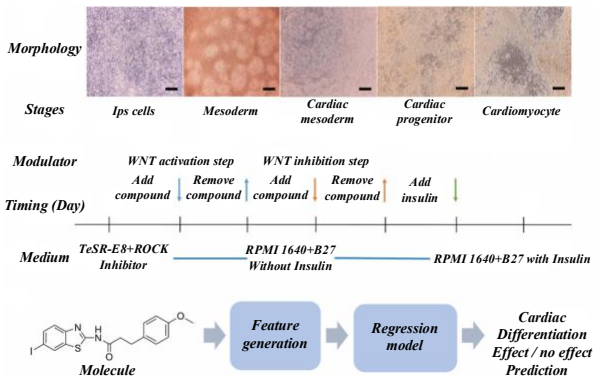

Fig. 1: Skin disease management model

Applying these methods to clinical treatment can improve the efficiency of diagnosis and treatment. In addition, in this process, it also promotes the study of biomolecules. This study may reveal more about the role of molecular kinematics and cytoskeletonics in the pathophysiology of bullous skin disease, and how mechanical stimulation in cells participates in the course of the disease and its diagnosis and treatment. In addition, understanding the elasticity of cell membranes and cell adhesion mechanics in the context of bullous skin disease is helpful in formulating more targeted diagnosis and treatment strategies.

A total of 60 patients participated in the study, including 32 men and 28 women; the age range is 25 to 70 years old, with an average age of 48.5 years old, and the participation period is 12 months from January 2022 to December 2022. Patients

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are recruited from a hospital, which is a third-level care center specializing in skin diseases. According to clinical and laboratory results, all the patients included in the study were diagnosed with herpes-like skin diseases, especially pemphigus vulgaris vulgaris and lobar pemphigus. The diagnostic method combines clinical examination, skin biopsy and direct immunofluorescence detection, and additionally uses ddPCR technology to quantify the autoantibody level in the patient's serum, which not only further supports the diagnosis, but also provides insight into disease activity.

The argument of this study is based on serious clinical infection. Previous dermatological studies have shown that mixed infection is a common phenomenon, accounting for 18.52% of all cases [25]. Among these mixed infection cases, as many as 42.07% (146 cases) have fungal infections, among which "fungal and bacterial" mixed infections are the most common (63.4%), "fungal and parasite" infections account for 25.6%, and combined infections of fungi, bacterial and parasites are less common (11%). The complex distribution of pathogens, especially the high proportion of fungi in mixed infections, puts forward extremely high requirements for the accuracy of diagnostic techniques. In order to meet this challenge, this study uses digital droplet PCR technology to accurately identify mixed infections, and evaluates its diagnostic effect by drawing the subject's working characteristics (ROC) curve. As shown in Fig. 2, the analysis results directly respond to the epidemiological data mentioned above. The left figure shows that ddPCR exhibits excellent diagnostic ability for detecting different pathogen combinations (parameters K1-K5), with all ROC curves significantly deviating from the diagonal. Its high area under the curve (AUC) confirms the excellent discriminatory ability of the technique. The right figure further validates the stability of key parameters (K3, K4, K5) within a wider range of false positive rates. DdPCR technology can efficiently and accurately identify the most common mixed infection patterns in clinical practice, providing key decision-making basis for early and precise antimicrobial therapy within the framework of "comprehensive nursing", and is expected to directly improve the infection management outcomes of patients with bullous skin disease.

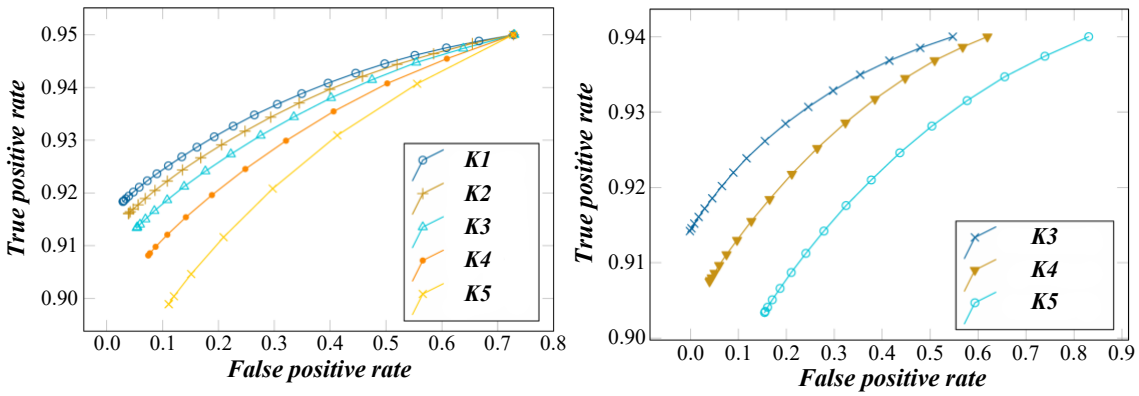

Fig. 2: Mixed infection data

A total of 60 patients were randomly assigned to the experimental group (n = 30) and the control group (n = 30). The experimental group received comprehensive care and ddPCR technology to monitor the levels of autoantibodies, including 17 males and 13 females, with an age range of 2768 years and an average age of 47.5 years, while the control group received standard medical care and routine care without ddPCR monitoring, including 15 males and 15 females, with an age range of 2570 years and an average age of 49.2 years. According to the analysis of LFD and PCR detection data in Fig. 3, the incidence of bullous skin diseases was the highest from June to September each year, during which a total of 367 cases were recorded, accounting for 62.41% of the total number of fungal skin diseases in the year, and the number of cases gradually increased from February to July (120 cases, accounting for 20.41%), and then gradually decreased from August to January of the following year, clearly showing the seasonal incidence trend of bullous skin diseases.

Digital droplet PCR (ddPCR) technology plays a key role in the treatment of bullous skin diseases in integrated care combined with digital droplet PCR technology. ddPCR technology is based on the principle of dividing a PCR reaction into tens of thousands of tiny droplets, each of which becomes an independent reaction unit for absolute quantification of the nucleic acid of interest. In the treatment of bullous skin disease, ddPCR testing of patient samples can accurately identify genetic mutations related to the disease. For example, the occurrence of bullous skin disease is often closely related to the mutations of certain key genes such as BP180, Dsg1/Dsg3, etc. ddPCR technology can capture subtle changes in these genes with high sensitivity and specificity. From the perspective of molecular mechanics, genetic mutations can change the

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molecular structure of proteins, which in turn affects their biological functions. Accurate detection of genetic mutations through ddPCR technology can help doctors understand the progress of the disease at the molecular level, thus providing a basis for personalized treatment plans. For example, for specific gene mutations, targeted drugs can be selected for precision treatment to block abnormal molecular signaling pathways to achieve the purpose of treating diseases. In terms of biomechanics, the mechanical properties of the skin, as the largest organ in the human body, are essential for maintaining normal physiological functions. The skin of patients with bullous skin diseases is abnormal in structure and function due to genetic mutations, and the mechanical properties are reduced, and symptoms such as blisters and breakage are easily occurring. ddPCR technology provides a dynamic view of the effects of disease on the biomechanical properties of the skin through continuous monitoring of genetic mutations. Based on this information, doctors can adjust treatment strategies in time at different stages of the disease, such as strengthening skin care and supportive treatment when the mechanical properties of the skin are rapidly declining, preventing the occurrence of complications, and effectively realizing the monitoring and treatment optimization of bullous skin diseases.

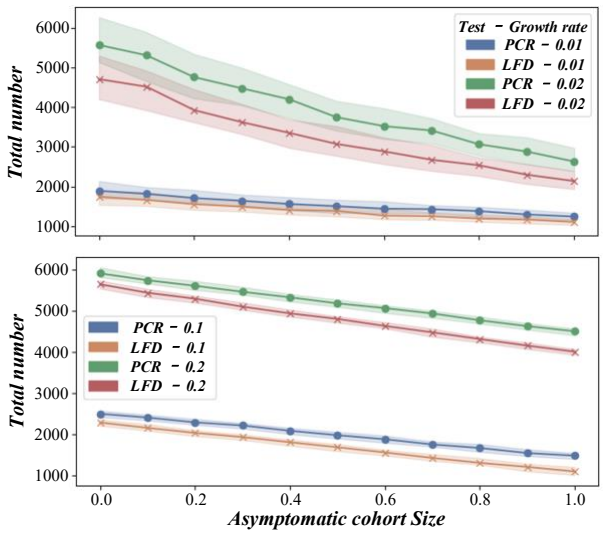

Fig. 3: LFD and PCR test

Development and Clinical Application of Bullous PMA-ddPCR Detection Technology Based on Comprehensive Nursing

Strain Detection Method Based on Comprehensive Care

Based on the framework of integrated care, we have successfully demonstrated a significant correlation between the technology and the detection of bullous pathogens in clinical bullous pathogens using an innovative bullous PMA-ddPCR detection technology supplemented by QuantaSoft software for data analysis. The PMA ddPCR detection technology for bullous skin diseases eliminates dead bacteria interference through PMA treatment, and combines ddPCR to achieve absolute quantification of live bacterial DNA, which is a specific high-precision molecular diagnostic technology for bullous skin diseases. By precisely controlling the treatment conditions of PMA (azide DNA dye), we are able to effectively distinguish between live and dead DNA, and then accurately determine the absolute concentration of bullous pathogens using the high sensitivity of ddPCR and the powerful analytical power of QuantaSoft software. Experimental results show that the technology is not only highly consistent with clinical diagnostic criteria, but also shows higher sensitivity in detecting pathogens at low concentrations.

In the experiment, the bullous standard strain (ATCC27562) first needs to be stored in 20% (v/v) glycerol cryopreservation solution at −80 ℃ in refrigerator. During activation culture, it should be placed in sterilized 2216E liquid medium, which can not only ensure the long-term preservation of the strain, but also effectively carry out activation culture for experimental use.

The core amplification targets of ddPCR are designed around disease mechanisms and efficacy evaluation, mainly including two categories: one is the encoding genes of autoimmune related antibodies (RNA level), DSG1, DSG3, COL17A1, DST; the second is inflammatory pathway molecules, such as IL-6, TNF - α, CXCL8, NF - κ B1.

The counted bullous culture solution was heated in a 95 ℃. dry thermostat for 10 min and then cooled to prepare a thermal lethal bacterial solution. Take 200 μL of this bacterial solution and uniformly spread it on 2216E agar plate, and ensure that the bacterial solution is dispersed by rotating the coating rod horizontally. The plate was cultured at 37 ℃ for 48 to 72 h. If no colony grew, it was confirmed that the bacterial solution was completely inactivated and contained all dead cells. This process effectively evaluates the inactivation effect and ensures that the bacterial solution used in the experiment is inactive.

This study uses digital droplet PCR (ddPCR) technology to dynamically monitor the disease activity of patients with bullous Epidermal Disease (EB). EB is a hereditary skin disease characterized by increased skin fragility, blisters and epidermal peeling, which can occur with mild trauma. We used ddPCR to quantitatively analyze the expression level of multiple genes related to epidermal barrier function in the patient's skin sample. The results showed that before the clinically visible skin peeling event, the expression of skin barrier-related genes in EB patients was significantly reduced, indicating that the disease was about to enter the active period. Based on the molecular warning information provided by ddPCR, we promptly adjusted the drug management plan, strengthened the use of moisturizers and skin protectants on the basis of conventional treatment, and implemented personalized comprehensive nursing interventions. Nursing measures include providing systematic skin protection education to patients, guiding them to adjust their daily behavior, avoid friction and trauma, and developing skin care plans to reduce the risk of blisters. By integrating the predictive nursing strategies based on ddPCR monitoring results, we effectively delayed the progression of skin peeling in patients, reduced the severity of clinical symptoms, and significantly improved their quality of life.

PMA Treatment and Optimization Under the Framework of Comprehensive Nursing

Integrated care is key in the treatment of bullous skin diseases in combination with digital droplet PCR. In terms of health education, the content involves the etiology, treatment, nursing and other knowledge of diseases, and is carried out by holding lectures, distributing manuals, and pushing popular science content online, and the effect is evaluated by a questionnaire on the knowledge awareness rate. In terms of psychological support, because patients are prone to anxiety and depression, one-on-one psychological counseling and patient mutual support groups are adopted, and anxiety and depression self-rating scales are used. Personalized care is formulated according to the patient’s condition, age, etc., light patients guide daily activities and skin cleansing, heavy patients regularly turn over to prevent pressure ulcers, strengthen nutrition, develop an exclusive plan for each patient, and evaluate the effect according to skin condition and quality of life scales. Through these comprehensive care and evaluation, the treatment effect of bullous skin disease can be effectively improved.

In the treatment of dermatology, systemic glucocorticoids are the cornerstone drugs that induce disease remission, and are usually given oral treatment at medium to high doses according to the severity of the condition. To reduce the long-term risk of hormone use, early combination with immunosuppressants (such as azathioprine and mycophenolate mofetil) is a standard strategy, and these drugs are used as "hormone savers". For moderate to severe or refractory cases, rituximab (a biological agent targeting B cells) has become a key treatment option for pemphigus vulgaris and is increasingly being used for bullous pemphigoid. In addition, the treatment system also includes intravenous injection of second-line or adjuvant drugs such as immunoglobulin and dapsone, forming a step-by-step individualized plan.

In terms of nursing resources, standard nursing focuses on wound management and supportive care. This includes using sterile techniques to treat blisters and ulcer surfaces, and choosing appropriate modern dressings according to exudates to promote healing and reduce dressing pain. Pain management, nutritional support (especially for patients with oral involvement) and the prevention and monitoring of local infections and systemic sepsis are the core of nursing. Meanwhile, providing continuous health education and psychological support to patients and their families to help them cope with the challenges posed by chronic diseases is an essential part of standard care.

In order to optimize the treatment conditions of PMA (Propidium Monoazide) and completely inhibit the amplification of dead bacteria DNA without affecting live bacteria DNA, the experiment was carried out under the framework of comprehensive nursing, with special attention to the sterility of the operation, the fineness of sample processing and the accuracy of subsequent analysis [26, 27]. Firstly, high concentration (2.65 × 108 CFU/mL) of pure culture bacterial solution was used as the experimental material to ensure the standardization of experimental initial conditions. Under the guidance of comprehensive care, a range of PMA working solutions of different concentrations are formulated, which are precisely prepared in a strict sterile environment to reduce any potential contamination. Under the comprehensive nursing framework, PMA processing and optimization involve regular inspections, maintenance, and upkeep of medical equipment to reduce equipment failure rates and the probability of accidents.

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Treat live bacteria with PMA and heat-lethal bacteria solution and culture them in a dark environment. At this stage, strictly control the ambient light and avoid non-specific light activation. Then, the bacterial solution was exposed with an LED blue light panel with an emission wavelength of 465nm. The best PMA concentration is determined by comparing Ct values under different PMA concentrations [28, 29]. On the basis of determining the optimal concentration, the effect of different light hours on the Ct value of thermally inactivated bacterial solutions is further evaluated to determine the optimal exposure time. The whole experimental process strictly follows the steps shown in Fig. 4 and is implemented within a comprehensive nursing framework to ensure the standardization of the operation and the repeatability of the results. In addition, the ddPCR technology used in the experiment can detect trace nucleic acid molecules in patient samples with high sensitivity, providing a key basis for early diagnosis and dynamic monitoring of diseases [30]. The whole experimental process strictly follows the specific operation shown in Fig. 4. The process begins with the addition of internal indexes and primer pairs to the target DNA molecules, which is the basis for subsequent high throughpult, multiple detection and accurate traceability. Then, the index sample was divided into a large number of independent droplets for PCR amplification. This physical separation effect greatly reduces the inhibitory interference in the complex background and realizes the "digital" absolute quantification of the target nucleic acid template. Its sensitivity is sufficient to detect low load potential infections or small biomarker fluctuations in BP patients. After sequencing, the amplification product is decoded and error corrected using pre-embedded index information to effectively distinguish the source of the amplification product and correct errors introduced by PCR or sequencing, ensuring that the final target data obtained has extremely high fidelity. Within the comprehensive nursing framework of BP, this technology can accurately quantify the dynamic changes in pathogen load or specific immune markers before and after treatment intervention. It is fully implemented under the framework of comprehensive nursing, ensuring the experimental results' reliability and repeatability and supporting medical diagnosis and disease monitoring.

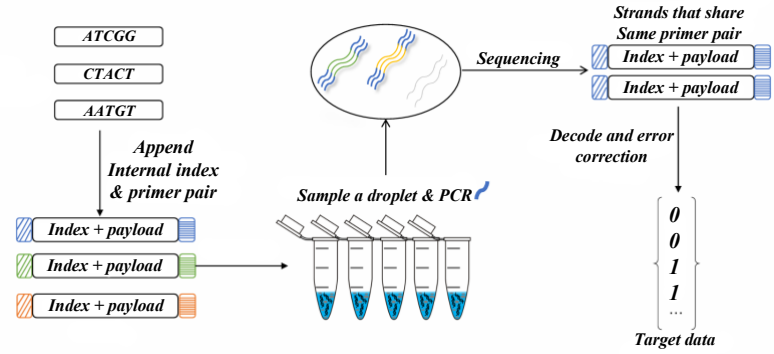

Fig. 4: PMA treatment model

In order to more widely validate the application value of ddPCR results in nursing practice, we also conducted a statistical analysis to compare the nursing outcomes of patients who received guidance based on ddPCR results with those who did not receive such guidance. The results showed that patients receiving ddPCR-guided nursing showed significant improvements in wound healing speed, infection rate control, medication compliance, and other aspects. This discovery further strengthens the role of ddPCR technology in optimizing nursing plans and improving nursing quality.

A total of three genes were detected using ddPCR, namely desmogenin 1 (DSG1), desmogenin 3 (DSG3), and anti-desmogenin autoantibody (anti-DSG). At the same time, we also provide specific forward and reverse primer sequences for each gene: the forward primer sequence is 5'-[sequence A]-3' for the DSG1 gene, the reverse primer sequence is 5'-[sequence B]-3';D the forward primer sequence for the SG3 gene is 5'-[sequence C]-3', and the reverse primer sequence is 5'-[sequence D]-3'.

The experimental procedure includes:

1. Incubating in the dark from light, packaging live bacteria and heat lethal bacteria solution into EP tube, adding appropriate amount of PMA solution and mixing, and incubating on ice in the dark from light for 15 min
2. Exposure, use a 465 nm LED blue light panel, and manually mix the EP tube every 5 min to ensure full exposure and avoid dead angles
3. DNA extraction, extract DNA in the dark immediately after exposure to reduce light interference

4. PCR amplification, using ddPCR technology based on qPCR, the extracted DNA was detected, and the optimal PMA concentration and exposure time were determined by analyzing Ct value. The whole process needs to control the lighting to ensure the accuracy of the experiment

In comprehensive nursing, ddPCR technology can be used to detect disease-specific biomarkers, so as to guide the nursing team to formulate personalized nursing plans. It can be seen from Table 1 that the average wound healing time (7 days) of the group of patients who received ddPCR-guided nursing was significantly shorter than that of the non-guided nursing group (12 days). This result reveals the positive effects of ddPCR monitoring on cell migration, proliferation and other repair kinetic processes from the perspective of cell mechanics. At the same time, ddPCR guided nursing controlled the infection rate at a low level of 5%, which was significantly lower than that of 15% in the control group. This highlights the key role of ddPCR technology in accurately identifying pathogens and distinguishing dead/living bacteria, making early targeted anti-infection treatment possible, and effectively inhibiting the damage of microorganisms to the mechanical integrity of the skin barrier. In terms of treatment compliance, the drug compliance rate of the ddPCR guidance group was as high as 90%, reflecting that health education and emotional support based on objective molecular data in comprehensive nursing significantly improved the treatment participation and self-management ability of patients. In summary, ddPCR, by providing highly sensitive molecular level data and synergizing with comprehensive nursing measures, not only improves clinical outcomes such as wound healing and infection control at the macro level, but also may reshape the disease process at the micro level by affecting cellular mechanical behavior and molecular pathways, providing evidence-based support for precise management of bullous skin diseases.

Table 1: Comparison of patient nursing effectiveness guided by ddPCR results

Nursing Guidance Type	Average Wound Healing Time (Days)	Infection Rate Control (%)	Medication Adherence Rate (%)
ddPCR-Guided Nursing	7	5	90
Non-Guided Nursing	12	15	70

Verification of Viable Bacteria Detection Capability of Digital Droplet PCR Technology Based on Comprehensive Nursing

To ensure the validity of the study results, the Consort reporting guidelines are strictly followed. In the randomization process, a random sequence is generated by computer, and a series of random numbers are generated according to preset rules through professional statistical software, which is used as the basis for grouping. In terms of grouping, after considering the characteristics of the study and the characteristics of the patients, a stratified randomization method was adopted to stratify the patients according to the severity of the patient's condition, age and other factors to ensure that the patients in each stratum were similar, and then the patients were assigned to the treatment group and the control group with comprehensive care combined with digital droplet PCR technology in each stratum, so as to balance the differences between the groups and improve the comparability of the study. In terms of allocation concealment, strict allocation concealment measures were implemented, and the generated random sequences were handed over to an independent third party for custody until the patient enrollment was completed, so as to avoid selection bias caused by the investigator's knowledge of the group information in advance. In the blinding setting, a double-blind design was adopted, both participants and investigators were blinded, participants did not know their own group, and the investigators did not know the grouping of patients when evaluating the treatment effect and collecting data, so as to effectively reduce information bias, ensure the objectivity and reliability of the research results, and improve the validity of the research results.

The suspension of bullous mixed bacteria containing different proportions of viable bacteria was designed, ranging from 100% viable bacteria to 0% viable bacteria, with a gradient of 20%. These mixtures were divided into two groups: one for the experimental group (PMA-qPCR group) and the other as the qPCR group. At the same time, a 2216E liquid medium mixture with the same concentration as viable bacteria was prepared as a blank control (2216E group). The experimental group and the blank control group were incubated according to the previously determined optimal PMA treatment conditions, and then the DNA of the treated bacterial solution was detected by bullous qPCR method. Notably, the qPCR panel was not treated with PMA and the results represented DNA amplification of total cells, both dead and living cells. The accuracy of the detection method for the detection of viable bacteria after PMA treatment was evaluated by comparing the *Ct* values of each group. The ddPCR technology greatly improves the sensitivity and accuracy of measurements by processing samples into microdroplets. It can detect extremely low concentrations of live bacteria, even single-copy nucleic acid molecules, which is of great significance for the early detection of live bacterial infections.

According to the relationship between sensitivity and p in Fig. 5, the Ct value of the thermally lethal bacteria group increased with the increase of PMA treatment concentration, and tended to be stable after reaching 100 μm, indicating that 100 μm was the initial concentration that completely inhibited DNA amplification of dead bacteria. Through One-way ANOVA test, it was found that PMA treatment of 10 μm to 180 μm had no significant inhibitory effect on PCR amplification of viable DNA ($P > 0.05$), and there was no significant difference in Ct value from that of untreated control group (0 μm).

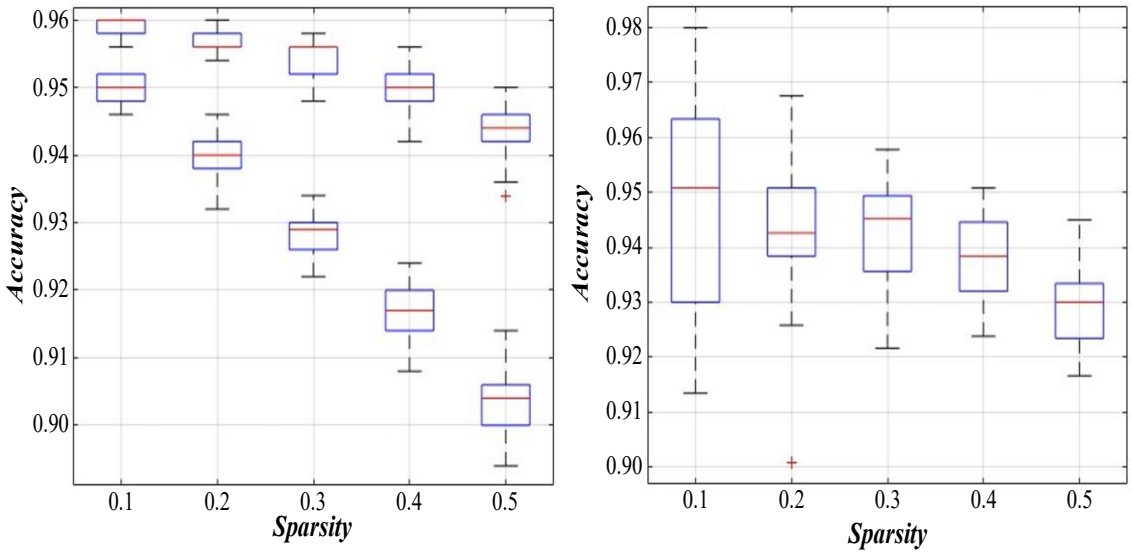

Fig. 5: Plot of sensitivity versus p

100 μm was determined as the best PMA treatment concentration. It can effectively inhibit the amplification of dead bacteria DNA without affecting the PCR process of live bacteria DNA. At the same time, it takes into account the control of experimental cost. It is an ideal choice to accurately distinguish the status of live and dead bacteria.

Fig. 6 shows the experimental results of FPL and SWT. Among them, the Ct values at 0 min were similar between the blank group (N group) and the group that added 0.1 μm PMA but did not undergo time exposure, confirming that the addition of PMA itself did not have a direct effect on the detection results of qPCR. In the 9 different PMA-treated groups setup, Ct values peaked at 10 min as the exposure time increased, and then slowly decreased as the exposure time was further extended. This phenomenon suggests that a 10-minute exposure time is the optimal time to inhibit the amplification of DNA-like DNA in dead bullae at a PMA concentration of 100 μm, as longer exposures may unnecessarily interfere with the detection of viable bacteria. Therefore, after comprehensive consideration, we have determined that 10 minutes is the most suitable exposure time, which can not only ensure the efficiency and specificity of PMA treatment, but also effectively avoid potential interference with subsequent live bacteria detection.

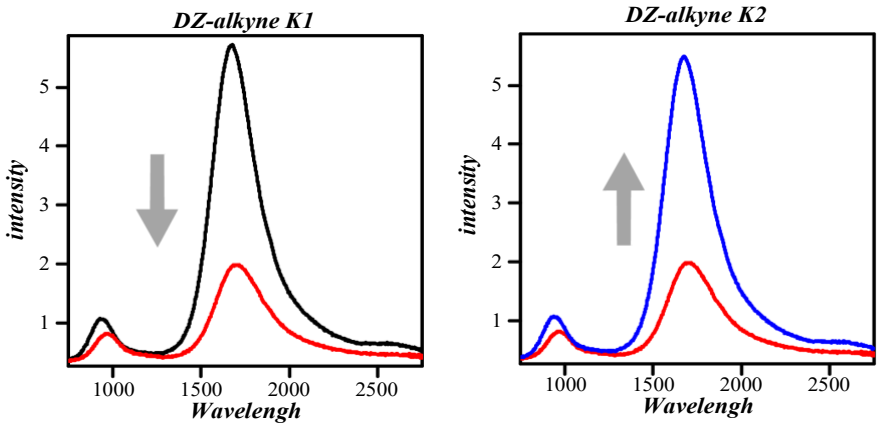

Fig. 6: FPL and SWT

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In order to verify the effectiveness of the new assay for the detection of live bacteria under optimal PMA (azide DNA dye) treatment conditions, a concentration of 0.5 μg/mL of PMA was used in the experiment. In the experiment, a bullous mixed bacterial solution containing different proportions of viable bacteria gradually decreased from 100% to 0%, and one group of bacterial solutions was treated with 0.5 μg/mL PMA for qPCR detection as the PMA-qPCR group. The other group was directly tested by qPCR without PMA treatment as the control qPCR group. At the same time, the same proportion of viable bacteria and treated with 0.5 μg/mL PMA was prepared with 2216E liquid medium as an additional control group (2216E group). By comparing the differences in the test results between the groups, the aim was to comprehensively evaluate the accuracy and reliability of the new method in accurately distinguishing between the status of live and dead bacteria.

Fig. 7 shows the absolute PL profiles of A and B, where the Ct values of conventional qPCR are not affected by the change in the proportion of viable bacteria in the template, which clearly points to the limitations of conventional qPCR methods that cannot distinguish between live and dead DNA. In contrast, when pretreated with 0.5 μg/mL PMA (PMA-qPCR group) and the control group (2216E group) with the same proportion of viable bacteria prepared with 2216E liquid medium and treated with PMA, the Ct values of both groups showed a significant downward trend with the increase of the proportion of viable bacteria, and the trends of the two groups were highly consistent, and the values were very similar. This result strongly demonstrates that the PCR method after PMA treatment can effectively detect live bullous bacteria and has the ability to accurately distinguish between live and dead bacterial DNA.

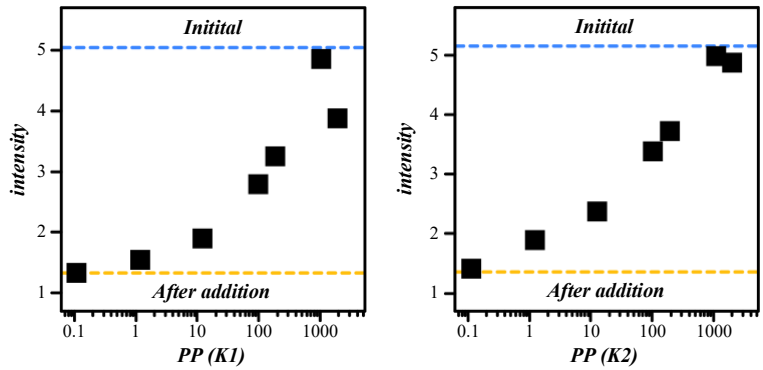

Fig. 7: A, B absolute PL spectra

Under optimized PMA treatment conditions, serial concentrations (6.625 CFU/mL to 1.325 × 107 CFU/mL) of bullous standard strains were detected by qPCR versus ddPCR with four replicates of each concentration. Fig. 8 shows the analysis of GC content and homopolymer function. Within the experimental range of the new qPCR (R2 = 0.9973) and ddPCR (R2 = 0.9965) methods, there is a strong correlation between the number of viable bacteria and Ct value or ddPCR copy number. Regression analysis showed that the LOD value of The PMA-ddPCR method was 29.33 CFU/mL, while the LOD value of the PMA-qPCR method was 1.14 × 103 CFU/mL. This indicates that the optimized PMA-ddPCR method is more sensitive in detecting bullous classes at low concentrations.

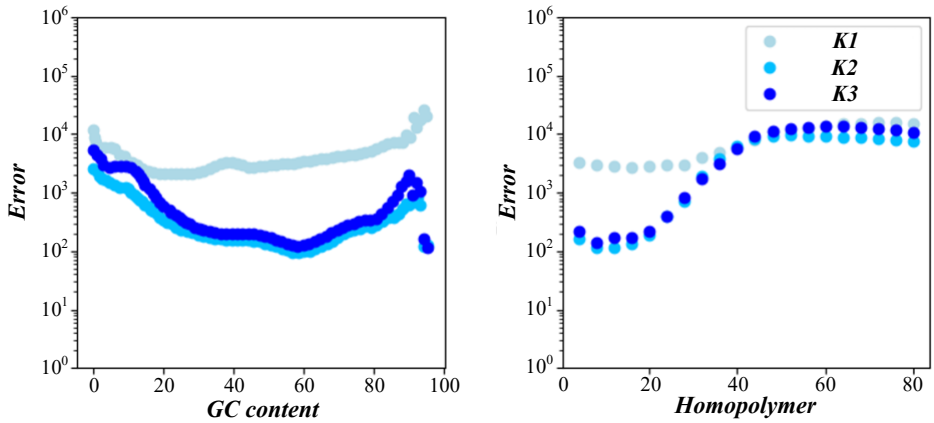

Fig. 8: GC content and homopolymer function analysis

Through the regression equation (PMA-qPCR: Y = −3.528X + 48.93; PMA-ddPCR: Y = 0.9368X − 2.033), Youden index and cut-off value analysis, the LOQ value of PMA-qPCR method in pure culture bacterial solution was calculated to be 1.29 × 103 CFU/mL, and the LOQ value of PMA-ddPCR method was 53.64 CFU/mL, as shown in Table 2. The optimized PMA-ddPCR method is more sensitive to the detection of bullous species at low concentrations.

Table 2: LOQ values of PMA-ddPCR method

Plate count (CFU/mL)	PMA-qPCR (*C* value)	PMA-ddPCR (Copies/uL)
15.96	28.308	6908.82
1596	33.192	607.902204
159,600	37.272	13.272
15,960,000	41.424	1.2
1,596,000,000	45.504	0.612
1,596,000,000	-	159.6

Specificity Test of qPCR Detection Method Based on Comprehensive Nursing Biomolecule-Based Digital PCR Reaction Mixtures

In order to further explore the detection techniques of biomolecules, especially genes, and to facilitate the application of relevant advanced mathematical methods in biological research, we designed an assay to verify the specificity of the qPCR method. The assay used a variety of control materials, including wild-type (WT) and mutant (MT1) of Campylobacter jejuni, related plasmids, DNA from Salmonella, E. coli and other bacteria, as well as chicken DNA and nuclease-free water, aiming to: (1) strictly verify the specificity of primers and probes to ensure that the qPCR method can accurately identify target genes, which is similar to the journal's pursuit of accurate identification and identification of biomolecules; and (2) to comprehensively evaluate the ability of the method to distinguish between different mutant subtypes of Campylobacter jejuni, thereby confirming its great potential for direct identification of mutant subtypes.

Both ddPCR and qPCR are based on the amplification of the target gene and quantification of the fluorescence value detected by the probe, and the primer and probe can be used universally. The ddPCR reaction system was 25 μL and contained 12.5 μL of 2 × PerfeCTa qPCR ToughMix UNG, 100 nm fluorescein, 1.2 μm primers, and 250 nm probes according to the manufacturer's recommendations. Under sterile conditions, 2.5 μL of purified genomic DNA was added to the prepared PCR mixture, mixed well and briefly centrifuged. Since the sensitivity of ddPCR is 2 orders of magnitude higher than that of qPCR, and it is more susceptible to DNA contamination of miscellaneous bacteria, the preparation of PCR mixture must be strictly aseptic to ensure the accuracy of the results.

Optimization of Reaction Conditions of qPCR Detection Method

In the primer concentration optimization test of qPCR reaction, by setting the primer concentration gradient (600 nM to 1800 nM), it was found that the Cq values of plasmids pWT-gyrA and pMT2-gyrA reached the minimum at the primer concentration of 1200 nM, and the Cq values of 1200 nM and 1500 nM were similar for pMT2-gyrA. In view of the combined consideration of minimum Cq value and cost-effectiveness, the optimal primer concentration for qPCR reaction was finally determined to be 1200 nM.

Through careful optimization, the optimal concentrations of primers and probes in qPCR method were determined to be 250 nM and 1200 nM, respectively. On this basis, the influence of annealing temperature in the range of 56 ℃ to 66 ℃ was deeply studied. The results showed that for Campylobacter jejuni WT and MT2 types, the Cq value was small and the non-specific response was low when the annealing temperature was 60 ℃ and 62 ℃; For the MT1 type, the annealing temperatures of 58 ℃ and 60 ℃ exhibit similar characteristics. Considering amplification efficiency, non-specific reaction and Cq value, 60 ℃ was selected as the optimal annealing temperature.

After optimization, it was determined that the optimal primer concentration for the qPCR reaction was 250 nM, the probe concentration was 1200 nM, and the annealing temperature was 60 ℃. Results of qPCR reactions performed under these

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conditions are shown in Fig. 9, Biological Constraints in assay images. Through digital droplet PCR technology, critical information can be effectively retained and optimized in reaction conditions.

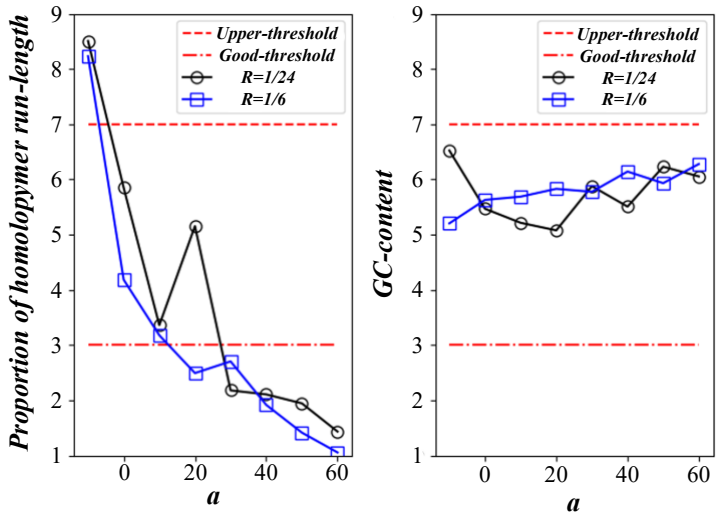

Fig. 9: Biological constraints in experimental images

Fig. 10 shows a comparison of ROC curves. The qPCR method can specifically detect Campylobacter jejuni, including wild-type (WT), mutant (MT1, MT2) and related plasmids. The method did not yield false positive results in negative controls of Salmonella, Campylobacter coli, Escherichia coli, Staphylococcus aureus, Clostridium perfringens, Pasteurella, chicken genomic DNA and nuclease-free water. This indicates that the qPCR method is particular, not only to detect C. jejuni directly but also to type it.

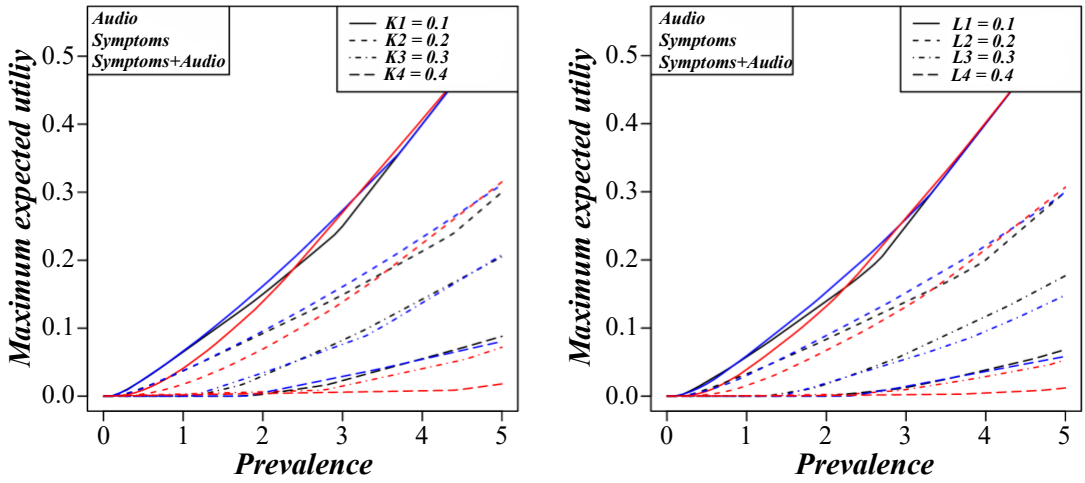

Fig. 10: Comparison of ROC curves

Fig. 11 shows the CoMix linear relationship when different Campylobacter jejuni plasmids (pWT-gyrA, pMT1-gyrA, pMT2-gyrA) are detected by qPCR method, specifically:

y = −3.5698x + 40.932, with a good linear relationship (R2 = 0.9956).

y = −3.4123x + 39.33, with a good linear relationship (R2 = 0.9995).

y = −3.4362x + 41.35, with a good linear relationship (R2 = 0.9995).

This indicates that the qPCR method shows high linear correlation in different plasmid detection and has good quantitative ability.

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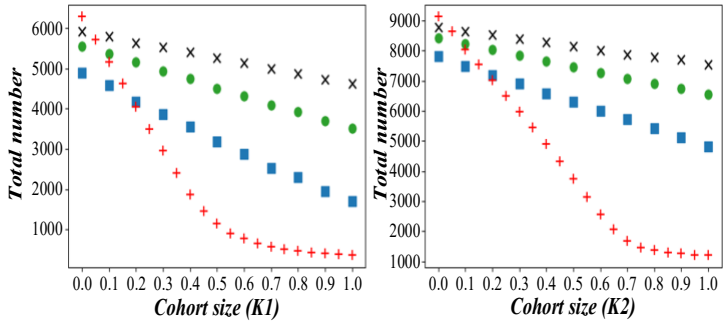

Fig. 11: CoMix linear relationship

Discussion

This randomized controlled trial included 60 patients with bullous skin disease (30 in each group). The experimental group received comprehensive nursing and digital droplet PCR (ddPCR) monitoring, while the control group received standard treatment for 12 weeks. The results showed that the expected grid rate of the experimental group was 92% (76% for the control group); ddPCR has better sensitivity (minimum detection limit of 29.33 CFU/mL, lower than the 1.14 × 10 ³ CFU/mL of PMA qPCR) and specificity for detecting pathogens and disease-related biomarkers, and can accurately distinguish live/dead bacteria, assisting in optimizing diagnosis and treatment; Patients who received ddPCR guided nursing had significantly better average wound healing time (7 days, compared to 12 days in the control group), infection rate (5%, compared to 15% in the control group), and medication adherence (90%, compared to 70% in the control group), and were able to dynamically monitor the expression of disease-related genes (such as DSG1 and DSG3).

This study focuses on the theme of "Comprehensive Nursing Combined with Digital Droplet PCR (ddPCR) Intervention for Bullous Skin Disease", and sets three validated clinical endpoints. Each endpoint measurement combines clinical indicators with molecular detection technology to ensure accurate and controllable measurement process. The core efficacy endpoint is the overall treatment response rate at 12 weeks, defined as a composite indicator of "skin lesion area reduction ≥ 50%, pruritus Visual Analogue Score (VAS) reduction ≥ 40%" and "ddPCR detection of gene copy numbers such as desmoglycan 1 (DSG1)/desmoglycan 3 (DSG3)/interleukin-6 (IL-6) returning to normal range". During measurement, standardized scales are used every 4 weeks to evaluate the area of skin lesions and the degree of itching, and QX200 is used every 6 weeks ™ The ddPCR system quantitatively detects the absolute copy number of disease-related genes in serum, and uses the reference range of healthy individuals as the criterion for judgment. The diagnostic efficacy endpoint focuses on the detection ability of ddPCR for pathogens and biomarkers. By detecting the vesicular standard strain (ATCC27562) and patient samples, the sensitivity (measured by the lowest detection limit (LOD) value, PMA ddPCR reached 29.33 CFU/mL, significantly better than qPCR's 1.14 × 10 ³ CFU/mL) and specificity (no false positives for wild-type/mutant (WT/MT) subtypes and miscellaneous bacteria detection of Campylobacter jejuni) were compared between PMA ddPCR and traditional real-time fluorescence quantitative PCR. The nursing outcome endpoints include wound healing time (average 7 days in the experimental group), infection control rate (5% in the experimental group), and medication adherence rate (90% in the experimental group), which are statistically analyzed through daily wound assessment, infection monitoring records, and medication check-in.

In the efficacy calculation stage, based on pre experimental data, the expected overall response rate of the experimental group was set to 92% and the control group was set to 76%. The test level α=0.05 and the test efficiency 1- β=0.8 were used as parameters. After calculating the sample size formula and considering a dropout rate of 20%, the sample size was finally determined to be 60 cases (30 cases in the experimental group and 30 cases in the control group). The grouping was conducted using a stratified randomization method, stratified by disease severity (mild/moderate) and age (<50 years/≥ 50 years). The random sequence was kept by an independent third party to achieve allocation concealment, and a double-blind design was used (both patients and outcome assessors were unaware of the grouping). In terms of multivariate analysis, SPSS 26.0 software was used, with "treatment response" as the dependent variable and age, gender, baseline skin lesion area, pathogen type, etc. as covariates included in the multivariate logistic regression model to correct for the influence of confounding factors; At the same time, one-way analysis of variance (ANOVA) was used to test the effect of PMA treatment concentration (10-180 μ M) on live bacterial DNA amplification, and the specificity of ddPCR detection was verified by

combining the receiver operating characteristic (ROC) curve (area under the curve (AUC)=0.98) to ensure the scientific and rigorous statistical results.

This randomized controlled trial has multiple limitations: the small sample size and strict inclusion standards lead to insufficient representation, and it is difficult to cover patients with different medical conditions, ages and potential diseases; although digital droplet PCR is accurate, it is expensive and has strict operational requirements; comprehensive nursing lacks standardized and quantitative indicators, and nursing people There are significant differences in the implementation of members, and it is difficult to accurately evaluate the effectiveness of a single measure. The following measures can be taken to solve the limitations of the experiment: expand the sample size and conduct joint multi-center trials, relax the inclusion criteria to include patients of different diseases, ages and complications, and improve sample representativeness through stratified sampling; optimize the application of digital droplet PCR, and cooperate with manufacturers to develop economical kits. , reduce the cost of batch testing, formulate operation manuals and train personnel to ensure consistent operation. Increase the correlation analysis of test results and long-term follow-up of patients' clinical symptoms; formulate the Standardized Operation Guidelines for Comprehensive Nursing, clarify the quantitative indicators of various measures, train and assess nursing staff, and supervise the implementation. Use the "single-factor variable method" to design subgroup experiments to accurately evaluate the effectiveness of single nursing measures.

Conclusion

Bullous skin disease is a serious threat to skin health and has long been a major challenge in dermatology. It requires in-depth exploration of biomolecular mechanisms such as protein structural mechanics, nucleic acid mechanics and protein interaction mechanics, and optimization of treatment strategies. Although traditional treatment methods have certain advantages, they are often accompanied by problems such as long treatment time and large side effects.

In recent years, with the rapid development of biotechnology, digital droplet PCR technology, as an advanced molecular biological detection method, has shown unique advantages in the treatment of bullous skin diseases. This technology has high sensitivity, specificity and high throughput, which can quickly and accurately detect genes related to bullous skin disease, help doctors accurately diagnose disease types, formulate personalized treatment plans, and significantly improve the treatment effect. At the same time, digital droplet PCR technology can also effectively monitor the recurrence of the disease and provide more comprehensive care for patients. In a clinical study involving 60 patients with bullous skin disease, the area of skin lesions decreased by an average of 70%. This not only shows the clinical application value of digital droplet Polymerase Chain Reaction (PCR) technology, but also further promotes the in-depth development of biomolecular research and cell mechanics research, covering the fields of cell membrane elasticity, cytoskeletal mechanics and internal force transmission.

ddPCR technology is of great clinical significance, especially in personalized treatment. Bullous skin disease is very complex, and each patient's symptoms and response to treatment are different. With its high sensitivity and accurate quantification, ddPCR technology can detect biomarkers related to bullous skin disease earlier and more accurately, such as BP180, Dsg1/Dsg3, etc. According to the results of these tests, doctors can keep abreast of the subtle changes in the patient's condition in the early stage of disease development, so as to adjust the treatment early. For example, when abnormal fluctuations in biomarker levels are detected, doctors may adjust the dose of immunosuppressants or change the treatment method. This early and precise adjustment of the treatment plan can help patients control the disease more effectively, reduce the occurrence of complications, significantly improve the prognosis of patients, and bring new hope and strong guarantees for the recovery of patients with bullous skin disease.

In the future, this research can be promoted from multiple dimensions: further optimizing digital droplet PCR technology, reducing detection costs, simplifying operational processes to enhance grassroots applicability, and exploring its association with subtypes and efficacy prediction of bullous skin diseases; Improve the comprehensive nursing system, develop personalized plans based on individual differences of patients, establish more accurate quantitative evaluation indicators for nursing effects, and optimize nursing processes through AI technology assistance; Conduct multi center, large sample long-term follow-up studies, including a wider population to verify the long-term effectiveness and safety of intervention plans, and conduct in-depth analysis of the impact of intervention measures on disease recurrence rates and patient quality of life, providing more sufficient evidence for clinical promotion.

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Ethics

This study has been approved by the hospital ethics committee. Prior to including all participants in the study, their written informed consent was obtained.

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